CARBON REMINERALIZATION AND STORAGE IN ESTUARINE WETLAND SEDIMENTS

Lisa A. Thatcher

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Approved by

Advisory Committee

Dr. Courtney T. Hackney

Dr. Lynn L. Leonard

Dr. G. Brooks Avery, Jr.
Advisor

Accepted by

Dr. Robert Roer
Dean, Graduate School

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ABSTRACT

Global warming has generated extensive research into the sources and removal mechanisms for the greenhouse gases carbon dioxide and methane. Recent research, in which sulfate was added to freshwater and methanogenic brackish sediments, showed that under sulfate-reducing conditions rates of carbon remineralization were more rapid. Therefore, less carbon should be stored in these systems under saline conditions.

In the current study, rates of carbon remineralization and storage were compared in Cape Fear River estuarine sediments with naturally-occurring spatial and temporal salinity variations. Trends were not evident along a salinity gradient for the percent organic matter content of sediments, sediment accumulation rates, or the total amount of carbon remineralized. These results show that there are no clear-cut patterns for biogeochemical parameters along a salinity gradient, likely due to variability in both primary productivity and remineralization rates. Although the quantity and quality of organic matter are generally thought to affect remineralization rates, rates in this study were controlled by sulfate concentrations. Most sediments responded to sulfate concentration increases with more rapid remineralization rates, showing that sulfate variations can potentially affect carbon storage in estuarine sediments.

An unexpected microbial process was observed during the experiments conducted for this study. The two main processes by which sediments are remineralized in the estuary are sulfate reduction and methane production. Sulfate reduction has generally been shown to out-compete and inhibit methane production via mutually exclusive biogeochemical zonation through competitive inhibition. However, for the first time the coexistence of the two processes at rates of similar magnitudes was observed.

A novel approach for calculating below-ground primary production within estuarine wetlands and carbon flux from estuarine wetlands, using sediment remineralization rates, ^{137}Cesium accumulation rates, and above-ground biomass measurements, is presented in this study. The largest amount of carbon fluxed from a site that contained the most above-ground biomass during the growing season. This study has shown that remineralization rates in estuarine sediments could potentially respond to sulfate variations on a short-term basis, supporting the idea that stored carbon could be released to the atmosphere during a rapid sea level rise.

ACKNOWLEDGEMENTS

My thanks first go to Dr. Brooks Avery. He was always there to provide me with guidance and a positive outlook regarding problems I came across during this project, he did his best to give me immediate feedback when needed, and he worked to get me to think "outside the box." I would also like to thank Dr. Courtney Hackney, who provided me with lab space and materials, an extra sulfide-smelling nose, iron discussions, summer work, and funny anecdotes about field work. My thanks also go to Dr. Lynn Leonard, who was there to answer many of my wetland questions and provided me with great suggestions regarding the thesis. Dr. Joan Willey also deserves thanks for always looking for student funding and for nominating me for a New Scholar award.

I would also like to acknowledge Jason Hall, Robert Lomax, and Katie Wimmer, who provided the transportation to get me to my sites on the river and the companionship to make it as enjoyable as possible. I would also like to thank Suzanne Sexton for her advice regarding the project and for helping me in my literature search. Nicholas Ottaway also deserves thanks for his part in helping to collect and analyze my samples. I am grateful to Alisha Renfro for allowing me to use her ^{137}Cesium data, Maverick Raber for helping me classify my samples, and my other friends in the grad school who made this experience rewarding. Thanks also go to the kind people in River Oaks and Echo Farms, who were nice enough to allow me to collect samples from their backyards.

Thanks also go to my boyfriend, Brent, who has always been there to listen to me when I needed to talk, to cook me dinner when I was hard at work, to make maps of my study sites, and basically to be one of my biggest supporters. The rest of my family and friends deserve thanks for supporting me and listening to me ramble on about this project.

DEDICATION

I would like to dedicate this thesis to my parents Mr. and Mrs. Lindell and Patricia Bonney, Mr. and Mrs. Thomas and Fay Thatcher, and my great aunt Mrs. Irene Balas. They have always supported me in whatever path I have chosen to pursue.

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INTRODUCTION

In recent years, global warming has become a major area of concern. During the last few thousand years, the planet's climate has remained rather stable; in contrast to the past (100,000 and 10,000 years B.P.), when the climate fluctuated more readily, e.g. local temperature increases of 5-10˚C over periods of only a few decades (Houghton et al. 2001). In the past 150 years, anthropogenic impacts on atmospheric carbon dioxide (CO_2) concentrations have risen by 25% since the pre-industrial period due to human combustion of fossil fuels and deforestation (Schneider 1989, Garrett 1992, Takahashi et al. 1992). This excess CO_2, along with increases in atmospheric methane (CH_4) gas, have been associated with an increase in the "greenhouse effect." Increased ocean temperatures, also caused by the "greenhouse effect," may lead to increases in sea level through the thermal expansion of oceans and glacial melting. Sea level is estimated to rise about 0.09 to 0.88 m over the next century (Houghton et al. 2001).

Some scientists think that the rate and duration of warming during the 20th century is larger than at any other time during the last 1000 years (Houghton et al. 2001). The prospect of global warming, caused in part by increases in atmospheric CO_2, has generated extensive research into the sources and removal mechanisms (sinks) for this greenhouse gas. Photosynthesis, or the conversion of atmospheric CO_2 into plant biomass, represents the largest annual biological removal mechanism for atmospheric CO_2 (Drake and Read 1981, Williams 1999, Houghton et al. 2001). If this biomass is respired (converted back to CO_2), then the CO_2 removed via photosynthesis is essentially put back into the atmosphere with no net removal. However, if the plant biomass is not

respired, and is eventually buried in sediments, long-term removal of atmospheric CO_2 can occur.

Respiration, or remineralization, is the breakdown of organic material to CO_2 and CH_4. It occurs most efficiently in aerobic environments. However, most waterlogged wetland sediments are anaerobic. Rates of remineralization are generally lower in anaerobic versus aerobic sediments (D'Angelo and Reddy 1999). Therefore, wetland soils are often able to store carbon in the form of dead plant biomass under these anaerobic conditions. In anoxic saltwater sediments, organic material is decomposed by the microbial process of sulfate reduction (SR) (Skyring 1987, Capone and Kiene 1988). This process occurs in the presence of the oxidant sulfate (SO_4^{2-}), a major ion in seawater (Millero 1996). In freshwater sediments, and brackish and marine sediments where sulfate concentrations have been depleted, anaerobic respiration occurs by methane production (MP), or methanogenesis (Capone and Kiene 1988, Day et al. 1989). Both SR and MP can occur in a wetland at the same time, although they are usually mutually exclusive (Lovley and Phillips 1987, Capone and Kiene 1988) due to biogeochemical zonation (Martens and Klump 1984, Lovley and Klug 1986, Kuivila et al. 1989).

Recent research into MP and SR involved the measurements of respiration rates of freshwater and methanogenic brackish sediments with and without the additions of seawater (Sexton 2002). The results of these experiments showed that the breakdown of organic material by methanogenesis is dramatically slower than by sulfate reduction. During sea level rise, when seawater intrudes into freshwater wetlands, the rate of carbon storage in these wetlands should decrease. In some cases, stored carbon could be rapidly released due to increased rates of respiration under sulfate-reducing conditions. In

addition to having an impact on global atmospheric carbon, the loss of biomass from wetlands could result in subsidence and the conversion of swamps into oligohaline tidal marshes (Hackney and Yelverton 1990) and finally into open water (Day et al. 2000, Sasser et al. 1986), as sedimentation rates may not be able to keep pace with sea level rise and subsidence rates (Hackney and Cleary 1987).

This study represents the first comprehensive examination of seasonal carbon dynamics in estuarine wetland systems. Although previous studies have shown the response of sediments to sulfate additions, none have shown the natural response of sediments to temporally-varying (seasonally and tidally) and spatially-varying *in situ* salinities. The main focus of this project was to compare the rates of carbon remineralization and storage in estuarine sediments with naturally-occurring salinity variations over different seasons and along a salinity gradient. One of the main goals of the current study was to determine if any spatial variations existed along a salinity gradient with respect to total carbon remineralized, percent organic matter, and sediment accretion rates. Also, a new approach for calculating below-ground primary production within an estuarine wetland and carbon flux from an estuarine wetland based on above-ground biomass measurements, respiration rates, and sediment accumulation rates from ^{137}Cesium data was developed; and the results are reported.

METHODS

Study Sites

Eight intertidal wetland sites along a salinity gradient in the lower Cape Fear River Basin, NC, were selected for this study. Five sites were part of the Army Corp of Engineers Cape Fear River Monitoring Project (CFMP) and included: Outer Town Creek (OTC), Inner Town Creek (ITC), Eagle Island (EI), Dollisons Landing (DL), and Black River (BR) (Hackney et al. 2002, 2003, 2004, 2005). Three sites were located in or near residential areas along the Cape Fear River and included: River Oaks (RO), Independence and River Road (IR), and Echo Farms (EF). Figure 1 shows a map of the sites and their locations along the Cape Fear River. Table 1 contains a list of the sites and their general characteristics. Salinities of the sites' sediment porewaters in the Cape Fear River Estuary were obtained from the CFMP and ranged from saline to intermediate.

Sample Collection and Preparation

Sediments were collected during 3 seasons: spring, summer, and winter. Spring Experiment samples were collected between May 7-June 3, 2003; Summer Experiment samples were collected between August 5-September 2, 2003; and Winter Experiment samples were collected between January 12-January 28, 2004. Above-ground biomass was collected only during the summer and winter experiments. Samples were obtained from the top 15 centimeters (cm) of sediment using a shovel and were stored in a closed plastic container in the laboratory prior to sample processing. To prepare the samples for incubation experiments, the sediments were homogenized in a blender for at least 3 minutes. Large objects were removed if they were too large to fit in the serum vials. A

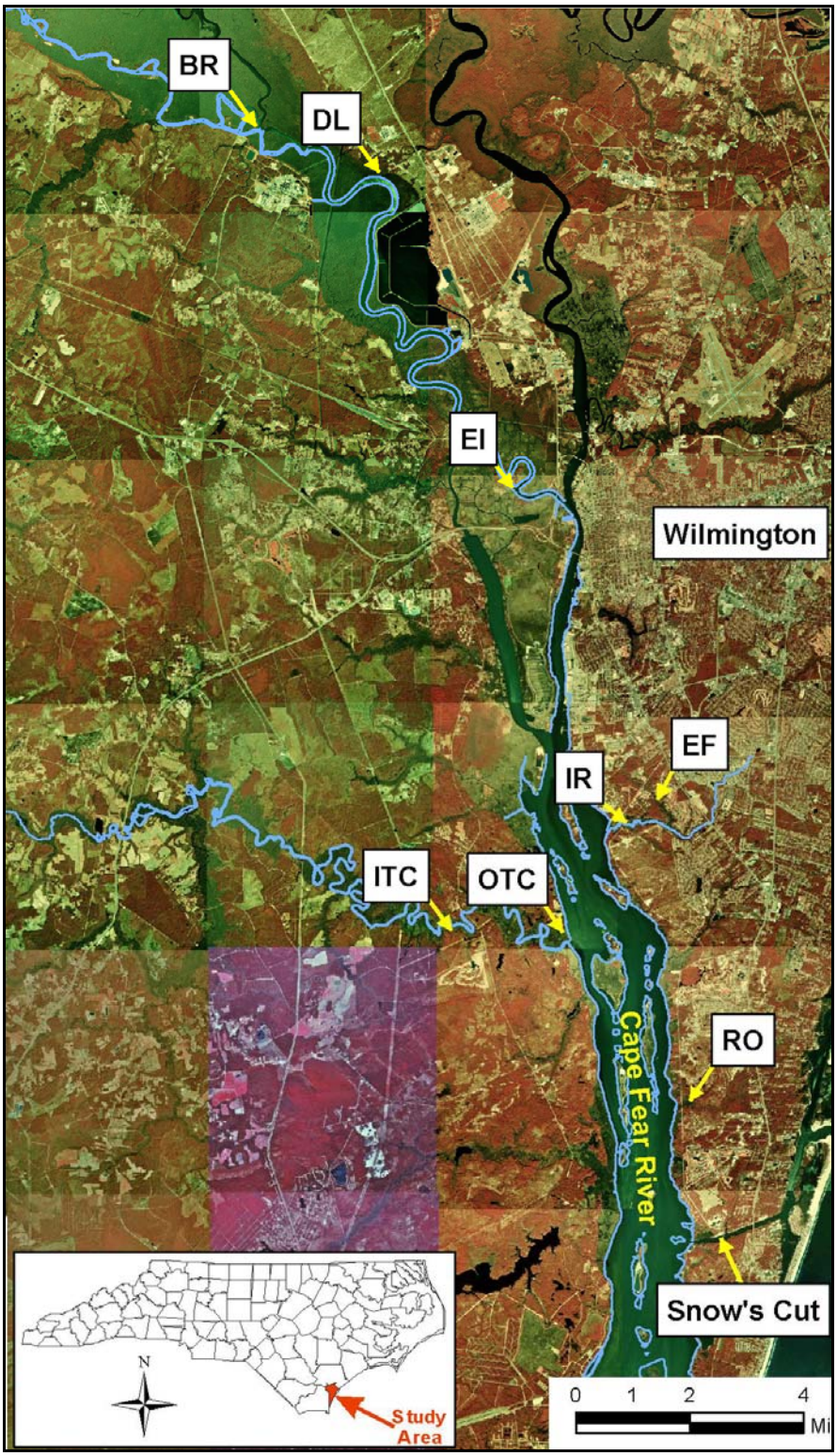


Figure 1. Site map. Map shows locations of the five Cape Fear River Monitoring Project sites (Hackney et al. 2004): Outer Town Creek (OTC), Inner Town Creek (ITC), Eagle Island (EI), Dollisons Landing (DL), and Black River (BR); and the three residential sites: River Oaks (RO), Independence and River Road (IR), and Echo Farms (EF).

Table 1. Site descriptions. Sites were regularly flooded with tidal waters at high tide and exposed at low tide. The salinity regime used to determine each site's wetland type was that used by the US Army Corp of Engineers (2004). Saline sediments were defined as having high salinities (20-40 ppt), brackish sediments had moderate salinities (0.5-16 ppt), and intermediate sediments had low salinities (0.5-8 ppt). Dominant vegetation listings were based on field observations at the time of sample collection and vegetation surveys in Hackney et al. (2004). Sediment descriptions were based on field and lab observations, and not any formal classification scheme.

Site	Hydrology	Type of Wetland	Dominant Vegetation	Sediment Description
River Oaks (RO)	Intertidal creek	Saline	Marsh – *Juncus romerianus*	Dark brown sediment, Muddy Dense root growth Sulfur odor
Outer Town Creek (OTC)	Intertidal	Saline	Marsh – *Spartina alterniflora*	Dark brown sediment, Muddy Dense root growth Sulfur odor
Independence & River (IR)	Intertidal creek	Saline	Marsh – *Spartina alterniflora*	Dark brown sediment, Clayey Dense root growth Sulfur odor
Echo Farms (EF)	Intertidal creek	Brackish	Swamp forest – *Typha* species across creek from site	Mudflat, Sandy mud Medium brown sediment Some detrital material
Inner Town Creek (ITC)	Intertidal creek	Brackish	Swamp forest – *Typha latifolia*, *Zizaniopsis miliacea*	Medium brown sediment Clayey
Eagle Island (EI)	Intertidal	Brackish	Marsh – *Spartina cynosuroides*	Reddish sediment, Muddy Dense root growth Sulfur odor
Dollisons Landing (DL)	Intertidal	Intermediate	Swamp forest – Canopy, *Polygonum* species, *Saururus cernuus*	Light brown sediment Muddy
Black River (BR)	Intertidal	Intermediate	Swamp forest – Canopy, *Ludwigia palustris, Polygonum* species	Light brown sediment Muddy

60 milliliter (ml) catheter-tipped syringe was used to inject 20 ml aliquots of the homogenized soil into 60 ml glass serum vials (Wheaton No. 223746). The RO sediment sampled in the winter contained a large number of roots and could not be blended. Therefore, individual pieces of the sediment were placed into the vials until 20 grams (g) had been added.

After sediments were added to vials, each was flushed with helium (He) to insure anoxic conditions, capped with a septum (Wheaton No. 224100-202), and crimp-sealed (Sexton 2002) with an aluminum cap (Wheaton No. 224183-01). The vials were stored inverted in the dark at a laboratory temperature of 23ºC. Triplicate vials were prepared for analysis at each time interval. After a specified incubation period, concentrations of methane (CH_4), carbon dioxide (CO_2), and sulfate (SO_4^{2-}) were measured.

Sample Preparation for Individual Experiments

Spring Experiment

The goal of the spring experiment was to determine rates of organic carbon remineralization under SR and MP conditions governed by *in situ* salinity and sulfate supply. Sediments were collected from all of the stations previously listed except for IR. (IR was added after spring to increase the number of sites sampled.) Blended sediment (20 ml) was added to each vial. A total of 147 vials were prepared.

Summer and Winter Experiments

The goal of the summer and winter experiments was to determine if carbon remineralization rates under MP and SR conditions were controlled by *in situ* salinity and

sulfate supply. The effect of sulfate additions on the sediment remineralization rates was also determined. Samples were collected from all stations for these two experiments. Vials (336) were prepared for each experiment and divided into treatment and control groups. To each treatment vial, 4 ml of anoxic deionized water (DI) plus 1 ml of anoxic 0.0704 M sodium sulfate (Na_2SO_4) solution were added to 20 ml of sediment, yielding a 2.8 μM SO_4^{2-} solution (Sexton 2002). To the control vials, 5 ml of anoxic DI were added to 20 ml of sediment. Above-ground biomass (AGB) was harvested from each site by cutting all of the plants located above the area (0.001767 m^2) from which sediments were taken. AGB was not taken from EF since it was a mudflat and contained no AGB.

Post-Incubation Sample Preparation for CH_4, CO_2, and SO_4^{2-} Analysis

Samples were sacrificed at predetermined time intervals and analyzed for CH_4 and CO_2 concentrations in all seasons and for SO_4^{2-} concentrations in spring and summer. These data were later used to calculate rates of MP, CDP, and SR. Five ml of 10% hydrochloric acid (HCl) were added to all of the vials to stop bacterial activity. The 10% HCl also converted all forms of CO_2 [$HCO_3^-{}_{(aq)}$, $CO_3^{2-}{}_{(aq)}$, and $CO_{2(aq)}$] to $CO_{2(g)}$, which is highly insoluble in water, therefore removing it to the headspace in the vial where it could be measured. HCl addition also shifted the equilibrium of any sulfide produced from sulfate reduction to the highly insoluble form hydrogen sulfide ($H_2S_{(g)}$), which was later removed by purging with an inert gas. This procedure prevented reoxidation of the H_2S to $SO_4^{2-}{}_{(aq)}$. After the HCl addition, vials were shaken for 1 minute to release highly insoluble methane and all forms of CO_2 to the headspace. Vials were also shaken to prevent diffusion constraints and anaerobic microsites from developing in the samples

(D'Angelo and Reddy 1999). Vials were stored inverted in a dark refrigerator (spring and summer experiments) or in a cabinet at room temperature (winter) until analysis.

Methane, Carbon Dioxide, and Sulfate Analytical Methods

Methane and Carbon Dioxide Analysis

Methane and carbon dioxide concentrations were measured for spring, summer, and winter samples. Prior to analysis, vials were shaken again briefly to remove any sediments from underneath the cap of the vial, so they did not clog the needle on the gas chromatograph (GC) gastight injection syringe (Hamilton 250 µl No. 1725). Twenty-five microliters (µl) of DI were injected into the vial to expel any sediment or rubber that may have clogged the GC syringe needle during insertion into the rubber stopper sealing the vial. A 100 µl sample from the headspace of each sealed vial was injected twice into a Hewlett-Packard HP6890 Series GC equipped with a thermal conductivity detector (TCD temp. = 250ºC, Oven temp. = 120ºC, Front Inlet temp. = 140ºC, Column 1-Helium carrier gas with flow rate = 30 ml/min). Both CH_4 and CO_2 were measured during each GC injection.

Methane concentrations were calculated from the peak areas compared to the peak area of a 1% methane standard (Scott Specialty Gases Mix 869). Carbon dioxide concentrations were calculated using a standard curve. CO_2 standards were prepared in serum vials and treated with HCl the same way as the samples: 20 ml of a specific sodium bicarbonate ($NaHCO_3$) concentration plus 5 ml of DI (simulating treatment) were added to each standard vial before sealing. Five ml of 10% HCl were added to each to

acidify the standards after sealing. CO_2 standard concentrations ranged from 50 µM to 5000 µM and had a least squared analysis R^2 value ≥ 0.99.

Sulfate Analysis

Sulfate concentrations were measured for spring and summer samples. After removal of crimp-seals and septa, vial contents were transferred to plastic 60-ml centrifuge tubes with caps. Within 30 minutes of being open to the atmosphere, hydrogen sulfide in the sediments (See Post-Incubation section above) was purged with a stream of helium for a period of 5 to 15 minutes or until no further sulfide odor was detected (Crill and Martens 1987, Sexton 2002). Samples were capped and centrifuged for 15 minutes at 9500 rpm to provide sediment-free porewater on top of the sediments.

Dilutions were made (1/51) to provide concentrations that were suitable for analysis via the ion chromatograph (IC). For the dilution, 100 µl of sample was removed from the supernatant in the centrifuge tubes and added to 5 ml of DI in 5 ml plastic auto-sampler vials with filter caps (Dionex Polyvial No. 038141). The vials were placed on the automated sampler (Dionex No. AS40) and were analyzed for sulfate according to protocol on a Dionex DX80 IC (Dionex 2001). Two injections were completed for each sample. Sulfate concentrations were calculated against a sulfate standard curve containing 4 standards: 0.1, 1, 10, and 100 ppm (equal to 2.82, 28.2, 282, and 2821 µM SO_4^{2-}). The CFMP's data was also used to report the seasonal sulfate concentrations and to interpret the changes in the sites that were due to sulfate concentration changes.

Calculation of Remineralization Rates

Remineralization rates were measured in control and amended samples for MP and carbon dioxide production (CDP) in spring, summer, and winter, and for SR in spring and summer. MP and CDP rates were determined by plotting the concentrations of CH_4 or CO_2 versus time, where the positive slope represented the CH_4 or CO_2 production rate (Avery et al. 2002, Sexton 2002). SR rate was determined by plotting the concentration of SO_4^{2-} versus time, where the negative slope equaled the sulfate reduction rate. Rates were obtained from the slopes of these lines calculated from a least squares analysis. A rate was considered to be significant if its R value, taking into consideration the appropriate degrees of freedom, fell within the 95% confidence interval ($P \leq 0.05$).

Control and amended rates were considered different where the error bars of samples (representing one standard deviation) did not overlap. To obtain standard deviations of the rates, three separate slopes were determined for each rate from the triplicate samples measured at each time point. Individual points in each slope were chosen randomly and were only used once. After the three slopes were obtained, an average and standard deviation of the rates were calculated (Avery and Martens 1999).

Calculation of Total Carbon Remineralized

Total carbon remineralized (TCR) was a measure of the total amount of carbon converted from organic matter to CH_4 and CO_2 in over 500 hours of sediment incubation. TCR at each site was calculated by first multiplying the rates of carbon dioxide and methane production by the elapsed time and then adding them together (i.e. Total Carbon Remineralized = MP Rate * Elapsed Time + CDP Rate * Elapsed Time). In some cases,

initial rates of respiration differed from final rates in both MP and CDP. If this occurred, the amount of carbon remineralized via each process was calculated separately for each time period and added together to obtain a total carbon remineralization value for the whole experiment. Although some incubation experiments were longer than others, it was possible to normalize them for comparison. In order to normalize results, the smallest incubation time reported during all three experiments (500 hours) was used for calculating TCR for all control samples.

The total amounts of carbon remineralized were reported for each site during spring, summer, and winter. The remineralization rates of some sites changed during the course of incubation. For example, at the beginning of incubation, some sites had rapid remineralization rates that later tapered off. Other sites had slow remineralization rates that rapidly increased after a certain length of time. If a noticeable rate change was observed, rates were broken into initial and final rate components for the TCR calculation. If rates were constant throughout the incubation, then rates were not broken into components for the calculation.

TCR was considered different for individual sites when the error bars (representing one standard deviation) in different seasons did not overlap. To obtain standard deviations, three separate totals were calculated for each site from the triplicate samples for each experiment. After the three totals were obtained, an average of the totals and a standard deviation were calculated (Avery and Martens 1999).

Sediment and Above-Ground Biomass Percent Organic Matter Analysis

Percent Organic Matter Content of Sediment

Organic matter (OM) content was determined using loss-on-ignition methods similar to those in Hargrave (1972). The goal of this organic content measurement was to quantify the amount of organic matter in the sediments of each site. In the spring experiment, the percent organic matter content was only determined for sediments. In summer and winter the percent OM content was determined for sediments as well as for the AGB.

Approximately 5-10 g of sediment from each site were placed in a pre-weighed crucible with a lid. Sediment samples contained all living and/or dead biomass found in the collected sediments. Large biomass was removed prior to placement in the crucible. Large biomass was considered to be any living and/or dead roots, etc., that were too large to fit in the crucible. The crucible containing the soil was reweighed. The sample was then dried to a constant weight in a 60-75°C drying oven for at least 3 days. It was reweighed, and the decrease in mass represented water loss. The sample was then ground with a mortar and pestle or electric grinder and transferred to a new, pre-weighed crucible with a lid. After the sample was placed in the new crucible, it was weighed again and placed in a combustion oven overnight at 550°C. The sample was reweighed, and the loss in mass represented a loss of organic content due to combustion (Hargrave 1972). Samples from each site were prepared in triplicate.

Above-Ground Biomass Bulk and Percent Organic Matter Content Measurement

The goal of this measurement was to quantify the amount of AGB located at each site and the percentage of organic matter it contained. These data were later used to calculate the amount of AGB that annually fluxed from the wetlands. AGB samples were obtained in the summer and winter from all sites except EF, which was a mudflat and contained no AGB. One AGB sample was collected from each site during summer and winter.

AGB samples were removed from pre-measured areas (0.001767 m^2) by cutting at the base of the plants. Samples were stored in zip-locking plastic bags and transported to the lab for analysis. Within 5 days of sampling, they were weighed to obtain bulk wet biomass measurements. The biomass was then cut into small sections and approximately 1-5 g of AGB were placed in pre-weighed crucibles with lids. The previously described loss-on-ignition procedure was used to determine the organic matter content of the AGB. Crucibles were prepared in triplicate for the organic matter analysis for each station.

The bulk wet measurements of above-ground biomass at all sites, percent OM content of dry weight AGB, and AGB OM measurement were determined at all sites during summer and winter. In order to determine if the OM content of the AGB was reflected in the sediments, the dry weight OM content of the AGB was divided by the dry weight OM content of the sediments for each site in summer and winter. The closer the ratio was to 1.0, the more likely that the OM content of the AGB was reflected in the sediments.

Statistics for Organic Matter Content

The t-test function in the statistical software Sigma Stat for Windows, Version 2.03 (SPSS Inc., ©1992-1997), was utilized to determine if the means of two groups were significantly different, assuming normality. Groups were considered significantly different where $P \leq 0.05$. In order to determine if there were statistical differences between the sediment OM in the sites seasonally, student's t-tests were performed three times for each site (spring vs. summer, summer vs. winter, and spring vs. winter). In order to establish if there were statistical differences between the AGB OM in the sites seasonally, student's t-tests were performed one time for each site (summer vs. winter).

Calculation of Below-Ground Primary Production and Carbon Flux from the Sediments

The goal of this calculation was to calculate the amount of below-ground primary production (BGPP) in the sediments as well as the amount of organic carbon flux from the sediments at the study sites. BGPP was considered new organic carbon incorporated into the sediments on an annual basis (i.e. respired plus accumulated carbon). Sediment accumulation rates were determined from gamma spectrometric measurements of cesium 137 (^{137}Cs), which is a thermonuclear bomb fallout isotope (Krishnaswamy et al. 1971, DeLaune et al. 1978, Alongi et al. 2004, Renfro 2004). ^{137}Cs data were obtained from Renfro (2004) to determine the annual sediment accumulation rates in EI, DL, and BR. Since remineralized carbon represented carbon from below-ground primary production that was later converted to CH_4 or CO_2, remineralization rates (carbon from MP and CDP) were added to the amount of organic carbon accumulated in the sediment to

determine the total amount of carbon produced in each site per year (Alongi et al. 2004 and references therein).

In addition to calculating below-ground primary production in the sites, it was possible to calculate the amount of carbon from AGB that fluxed from sediments. Carbon flux represented AGB that was produced within and removed from the system on an annual basis. Carbon flux was calculated by subtracting the amount of AGB present during winter from the amount of AGB present during summer, excluding any woody biomass (e.g. trees). Certain assumptions were made for these calculations. Bulk density was assumed to be 1.5 g/cm^3 (Capone and Kiene 1988). Respiration rates from the summer experiment were used in this calculation since the temperature at which this experiment was conducted (~ 23ºC) is within a few degrees of average *in situ* temperatures observed for the Cape Fear River Estuary during the year (Mallin et al. 2003). Annual respiration rates were calculated by assuming the summer rates occurred for 9 months since southeastern North Carolina has a relatively mild climate with only 3 cold months. Refer to Figure 2 for a generalized box model used in the carbon calculations.

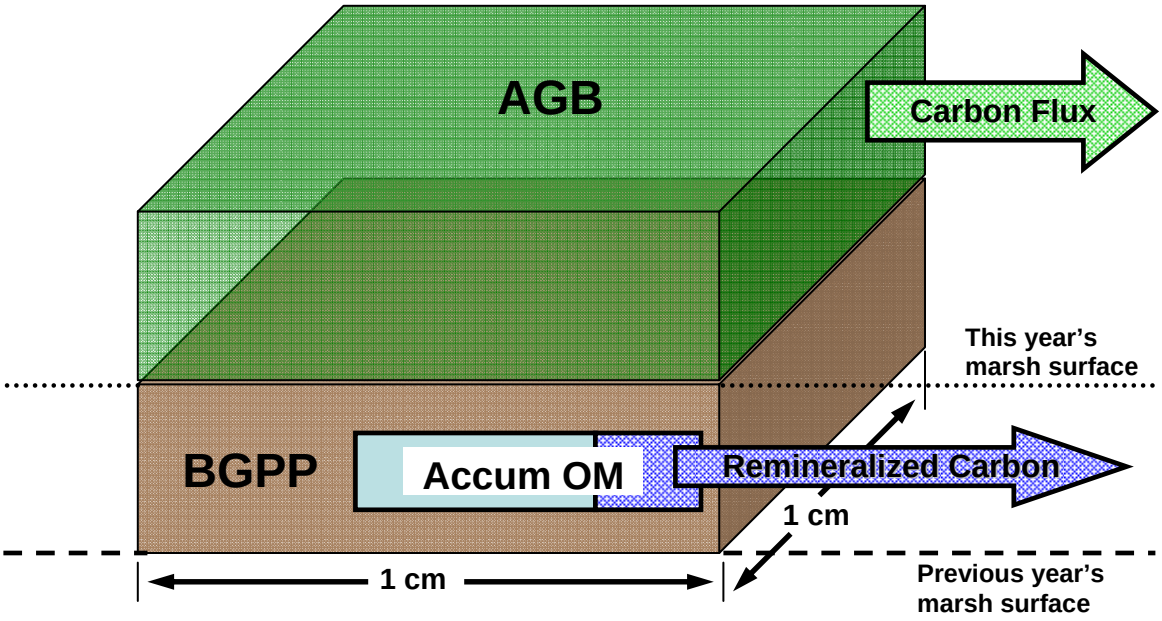


Figure 2. Generalized box model for below-ground primary production and carbon flux calculations. (Box consists of a 1 cm^2 area multiplied by the height of the annually accumulated sediments.) Accumulated organic matter (Accum OM) is the amount of organic material from below-ground primary production (BGPP) and sedimentation that is incorporated into the sediments. It is calculated using ^{137}Cesium data, predetermined percent OM values, and remineralized carbon measurements. Remineralized Carbon is the amount of organic material that is respired, measured from rates of CH_4 and CO_2 production data. Carbon Flux is the amount of carbon from above-ground biomass (AGB) produced within and removed from the system. (AGB does not include woody biomass.)

Below-ground primary production and the flux of organic carbon from the sediments were calculated by the following:

BGPP Calculation (g $C/m^2/yr$):

$$BGPP = [(SAR * SOM * 0.537) + (RR * SAR * 1.05E\text{-}4)] * 10000$$

Carbon Flux Calculation (g $C/m^2/yr$):

$$BC = BW * AGB\ OM * 0.358$$

Carbon Flux from Sediments = Summer BC – Winter BC,

where SAR = Sediment Accumulation Rate (cm^3/yr), SOM = Dry Weight Sediment Organic Matter Fraction, RR = Remineralization Rate (μM/hr), BC = Bulk Carbon (g $C/m^2/yr$), BW = Bulk Wet Above-Ground Biomass (g $biomass/m^2/yr$) during respective season, and AGB OM = Dry Weight Above-Ground Biomass Organic Matter Fraction during respective season. Factors included in the calculation are 0.537 = bulk density of 1.5 g/cm^3 * conversion of g OM to g Carbon (106 * 12 / 3550), 1.05E-4 = conversion from μM/hr to g $C/cm^3/yr$ to g $C/cm^2/yr$ (24 * 273.75 * 12 / 1000 / 10^6) where 273.75 days is approximately 9 months or ¾ of a year (summer season), 10000 = conversion from g $C/cm^2/yr$ to g $C/m^2/yr$, and 0.358 = conversion from g $OM/m^2/yr$ to g $C/m^2/yr$ (106 * 12 / 3550).

RESULTS

Initial Sulfate Concentrations

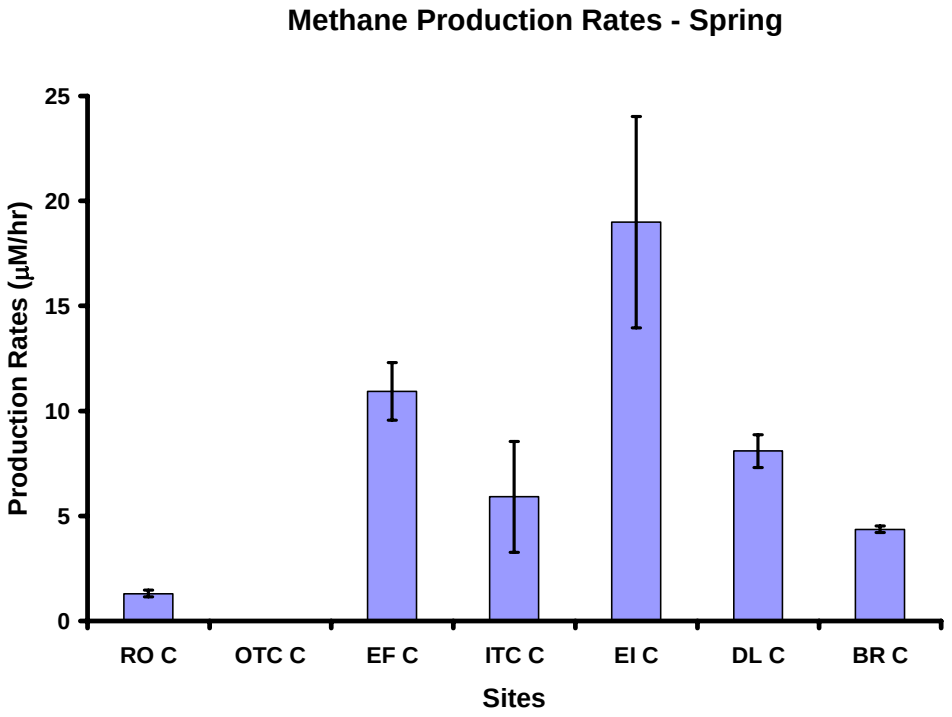
The initial sulfate concentrations of sediment porewaters from control samples during each experiment ranged from 83 μM to 11 x 10^3 μM in summer 2003.

Rates of Methane Production

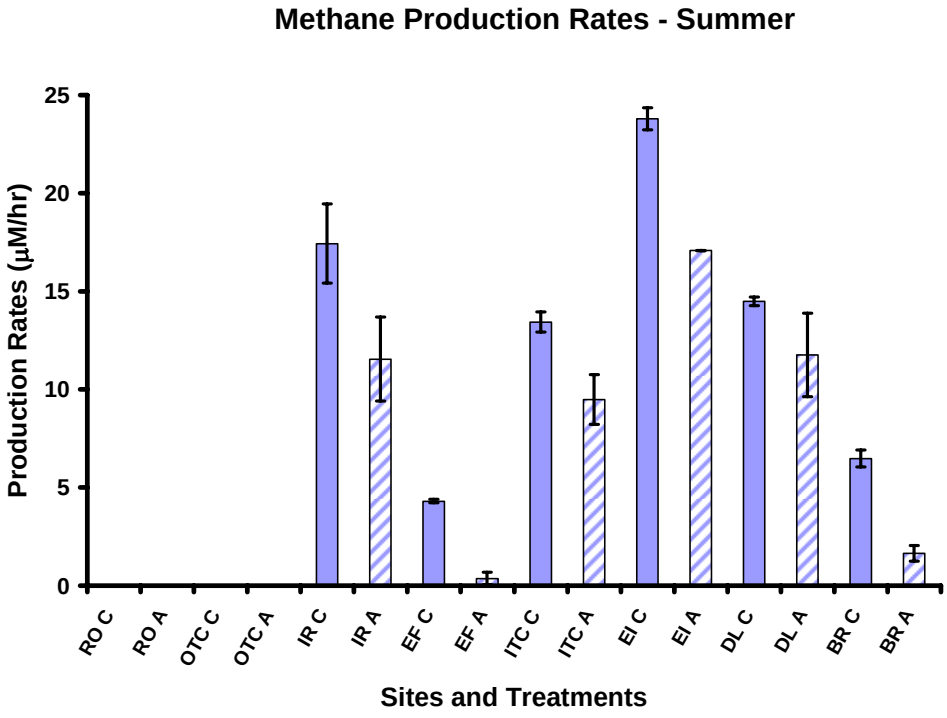
Rates of MP ranged from 1.3 to 19.0 μM/hr for control samples (Figure 3). During the spring, MP was observed in all sites except OTC, one of the most seaward and more saline sites (Figure 3a). In the summer when sulfate supply was relatively low, MP was observed in all sites except the amended OTC sample (Figure 3b). During the summer, all sites had control MP rates greater than amended rates, except in RO and OTC where there was no MP. During winter when sulfate concentrations were slightly greater, MP was observed in all sites except for OTC control and amended (Figure 3c). During the winter when MP was detected, more than half of the control MP rates were greater than amended rates. The remaining sites had no difference between control and amended rates.

Rates of Sulfate Reduction

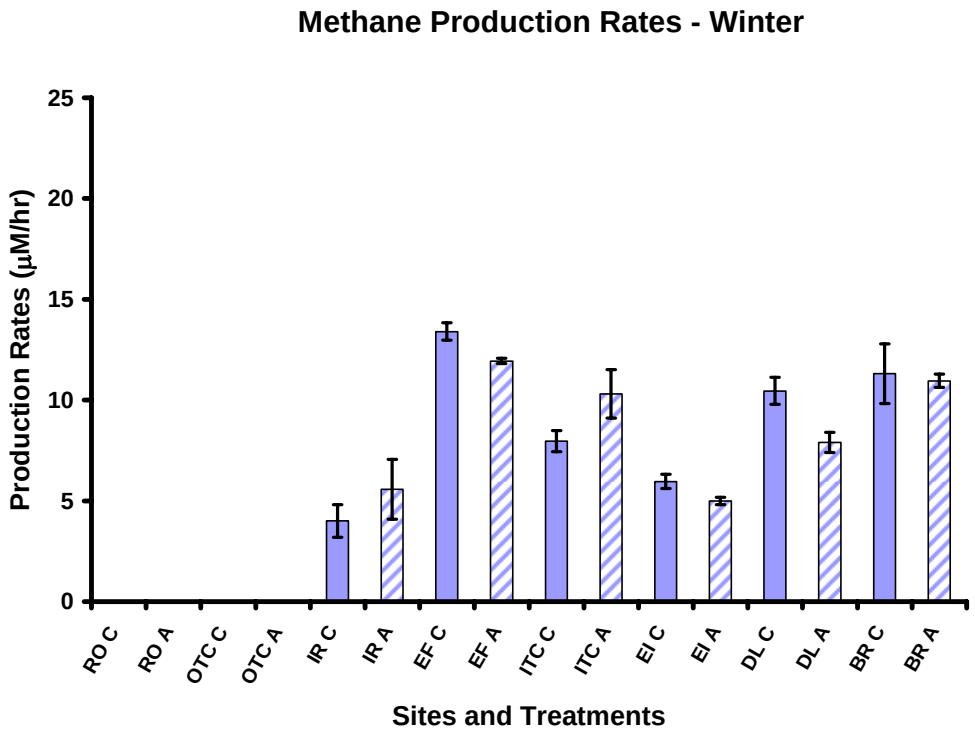
Rates of SR ranged from 1.4 to 38.5 μM/hr for control samples (Figure 4) from spring and summer. During the spring, SR was observed in every site except EF (Figure 4a). In the summer, even though sulfate supply was relatively low, SR was observed in all sites except for ITC control and BR control (Figure 4b). Most sites had higher rates


Methane Production Rates - Spring
Production Rates (µM/hr)
25
20
15
10
5
0
RO C
OTC C
EF C
ITC C
EI C
DL C
BR C
Sites

a.


Methane Production Rates - Summer
Production Rates (µM/hr)
25
20
15
10
5
0
RO C
RO A
OTC C
OTC A
IR C
IR A
EF C
EF A
ITC C
ITC A
EI C
EI A
DL C
DL A
BR C
BR A
Sites and Treatments

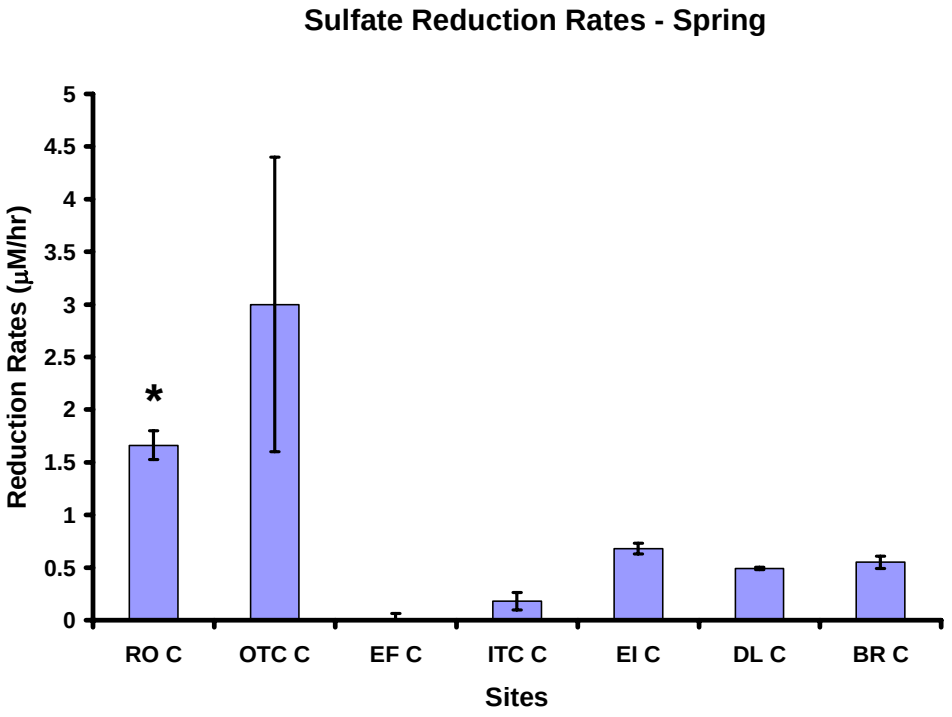
b.



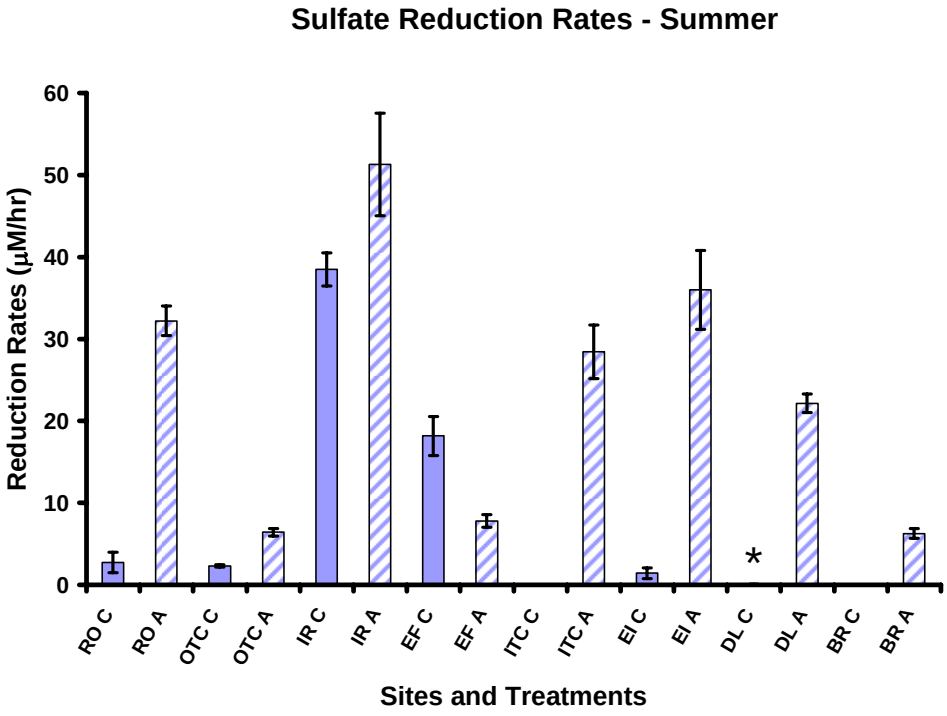
c.

Figure 3. Rates of methane production. Sites are arranged along a general salinity gradient, although no specific pattern was observed. Error bars represent one standard deviation of the rate. To obtain standard deviations of the rates, three separate slopes were determined for each rate from the triplicate samples measured at each time point. Individual points in each slope were chosen randomly and were only used once. After the three slopes were obtained, an average of the rates and a standard deviation were calculated. If no column is present, the rate at that site was zero. a.) Only control groups are shown for spring (there were no amended samples) and are designated with a "C." b, c.) Both control groups and treatment groups are shown for each site in summer and winter. Controls are designated "C" and are solid-colored columns, and amended samples are designated "A" and are striped columns.

Figure 4. Rates of sulfate reduction. Sites are arranged along a general salinity gradient, although no specific pattern was observed. Error bars represent one standard deviation of the rate. To obtain standard deviations of the rates, three separate slopes were determined for each rate from the triplicate samples measured at each time point. Individual points in each slope were chosen randomly and were only used once. After the three slopes were obtained, an average of the rates and a standard deviation were calculated. If no column is present, the rate at that site was zero. a.) Only control groups are shown for spring (there were no amended samples) and are designated with a "C." The asterisk (*) located over the rate of RO C indicates that this bar is 1/10 of the actual value of the rate. b.) Both control groups and treatment groups are shown for each site in summer. Controls are designated "C" and are solid-colored columns, and amended samples are designated "A" and are striped columns. The asterisk (*) located over the rate of DL C during summer indicates that SR did occur in this sample at a rate of 0.1 μM/hr, but it was too small to be observed on the graph. Note: Scales are different for each graph.


Sulfate Reduction Rates - Spring
Reduction Rates (µM/hr)
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
*
RO C
OTC C
EF C
ITC C
EI C
DL C
BR C
Sites

a.


Sulfate Reduction Rates - Summer
Reduction Rates (µM/hr)
60
50
40
30
20
10
0
*
RO C
RO A
OTC C
OTC A
IR C
IR A
EF C
EF A
ITC C
ITC A
EI C
EI A
DL C
DL A
BR C
BR A
Sites and Treatments

b.

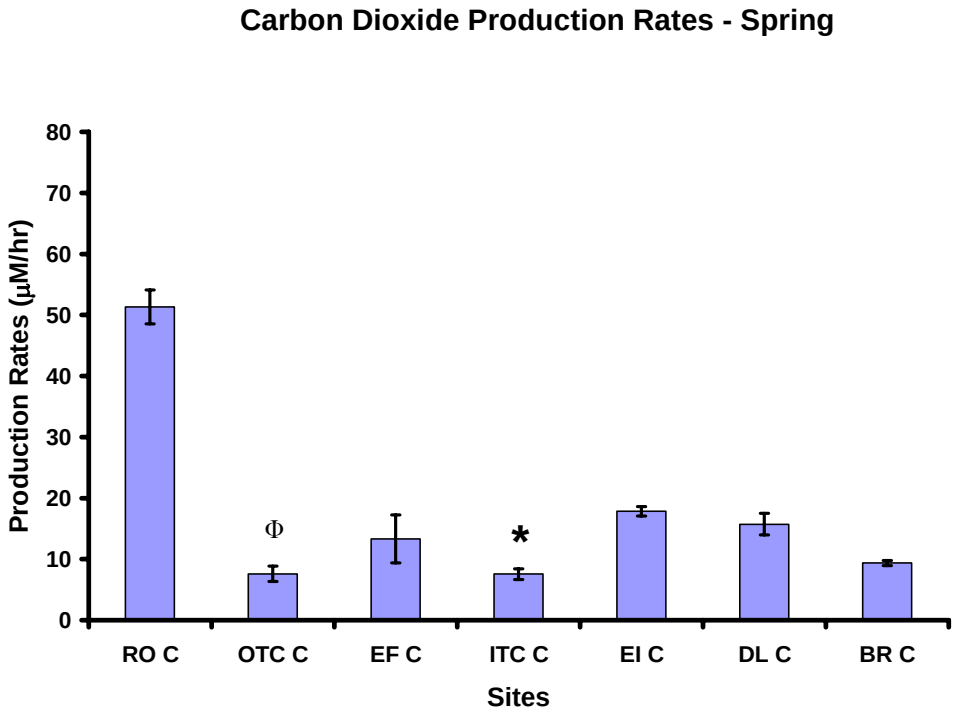
of SR in amended samples versus controls during the summer (7 out of 8). No correlation was found between SR rates and locations of sites along a generalized salinity gradient. An unanticipated microbial process was discovered that showed for the first time that MP can occur at the same time and approximately the same rate as SR in wetland sediments.

Rates of Carbon Dioxide Production

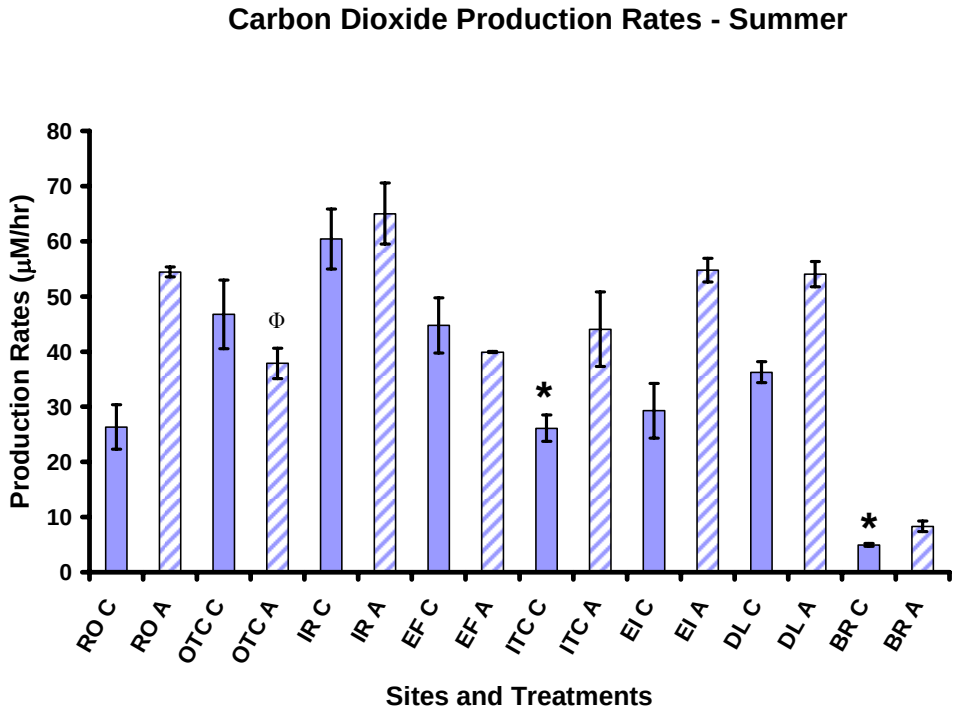
Carbon dioxide production rates ranged from 5.0 to 125.3 μM/hr for control samples (Figure 5). CO_2 production was observed in all sites during each experiment. During the summer, when sulfate concentrations were relatively low, more than half of the sites (5 out of 8) had greater rates of CO_2 production in the amended samples versus the controls (Figure 5b). During the winter, when sulfate concentrations were slightly greater, less than half of the sites (3 out of 8) had greater rates of CO_2 production in the amended samples versus the controls. The CDP rates in amended and controls samples of 3 sites were equal, and 2 sites had greater rates of CDP in the controls (Figure 5c).

Total Carbon Remineralization

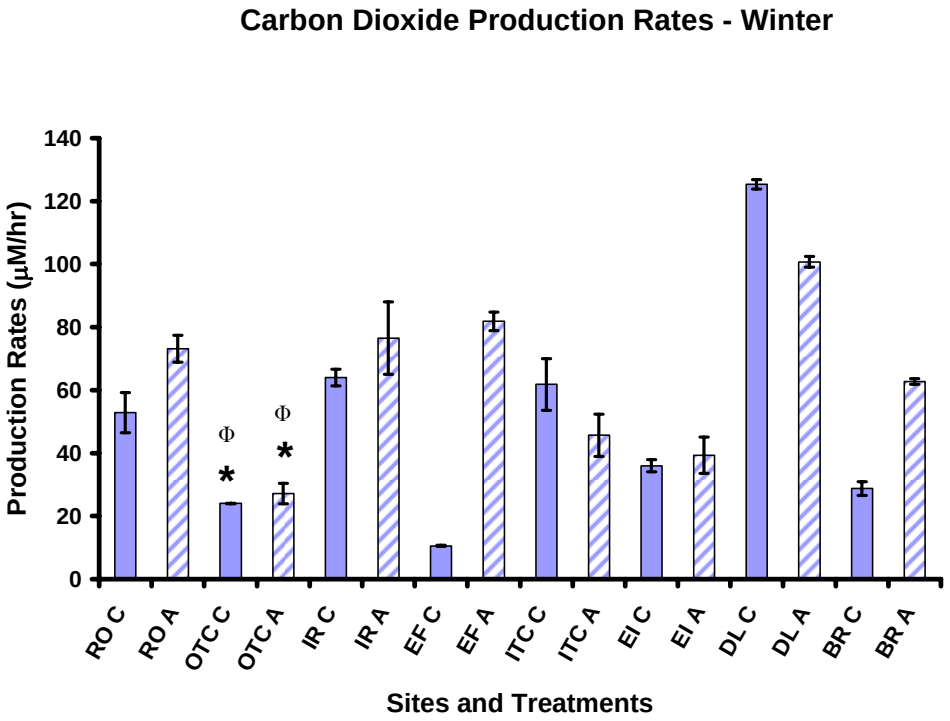
More than half of the sites had greater TCR in the winter than summer (5 out of 8). No trend was observed for the amount of carbon remineralized along the general salinity gradient (Figure 6).


Carbon Dioxide Production Rates - Spring
Production Rates (µM/hr)
80
70
60
50
40
30
20
10
0
Φ
*
RO C
OTC C
EF C
ITC C
EI C
DL C
BR C
Sites

a.


Carbon Dioxide Production Rates - Summer
Production Rates (µM/hr)
80
70
60
50
40
30
20
10
0
Φ
*
*
RO C
RO A
OTC C
OTC A
IR C
IR A
EF C
EF A
ITC C
ITC A
EI C
EI A
DL C
DL A
BR C
BR A
Sites and Treatments

b.



c.

Figure 5. Rates of carbon dioxide production. Sites are arranged along a general salinity gradient, although no specific pattern was observed. Error bars represent one standard deviation of the rate. To obtain standard deviations of the rates, three separate slopes were determined for each rate from the triplicate samples measured at each time point. Individual points in each slope were chosen randomly and were only used once. After the three slopes were obtained, an average of the rates and a standard deviation were calculated. If no column is present, the rate at that site was zero. An asterisk (*) located over a column indicates that no SR occurred in the sample during the experiment. A phi (Φ) located over a column indicates that no MP occurred during the experiment. a.) Only control groups are shown for spring (there were no amended samples) and are designated with a "C." b, c.) Both control groups and treatment groups are shown for each site in summer and winter. Controls are designated "C" and are solid-colored columns, and amended samples are designated "A" and are striped columns. Note: Scale is different on Graph c.

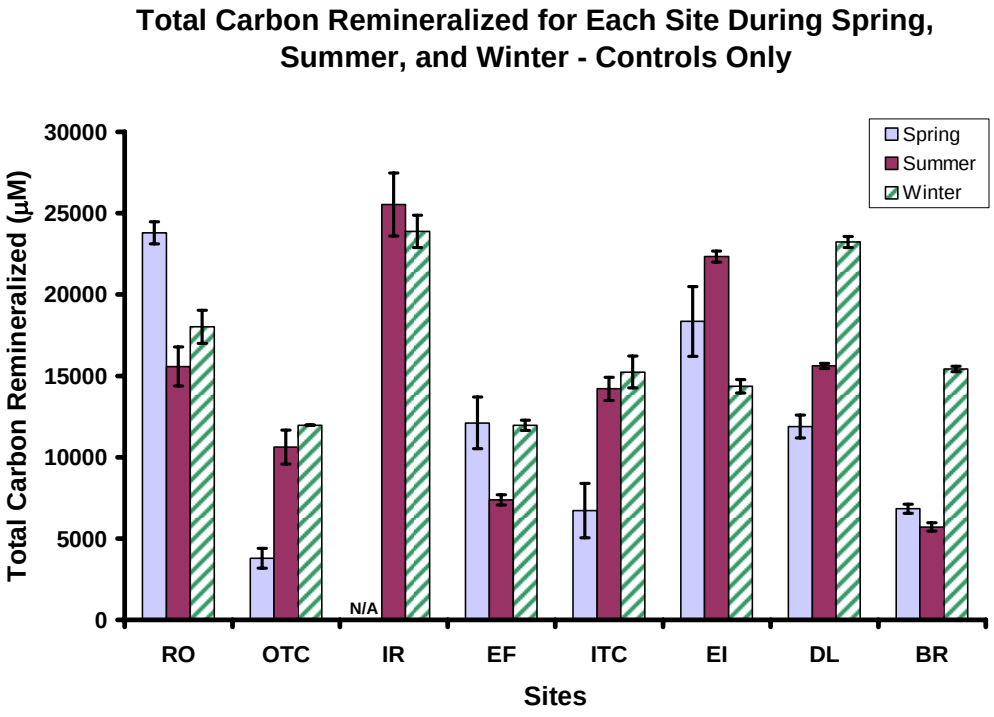


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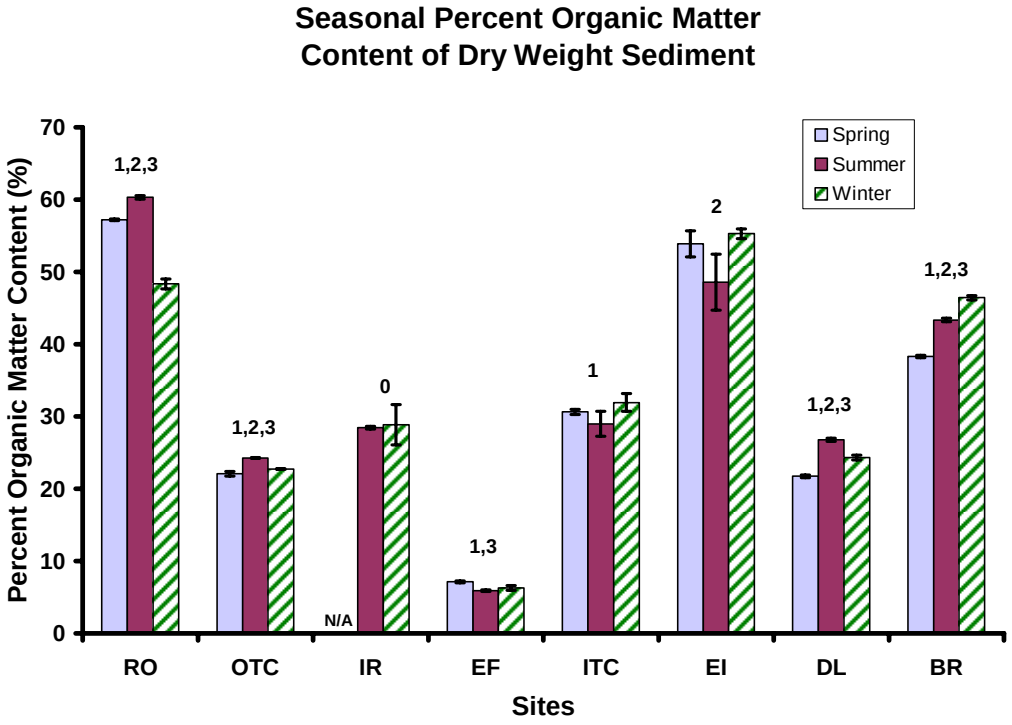
Figure 6. Total carbon remineralized for each site during spring, summer, and winter – controls only. TCR was calculated using an incubation time of 500 hours for each sample. Error bars represent one standard deviation. To obtain standard deviations of the totals of remineralized carbon, three separate totals were calculated for each site from the triplicate samples in each experiment. After the three totals were obtained, an average of the totals and a standard deviation were calculated. Samples were not collected from IR during the spring, so "N/A" (not applicable) is indicated.

Percent Organic Matter Content of Sediments and Above-Ground Biomass

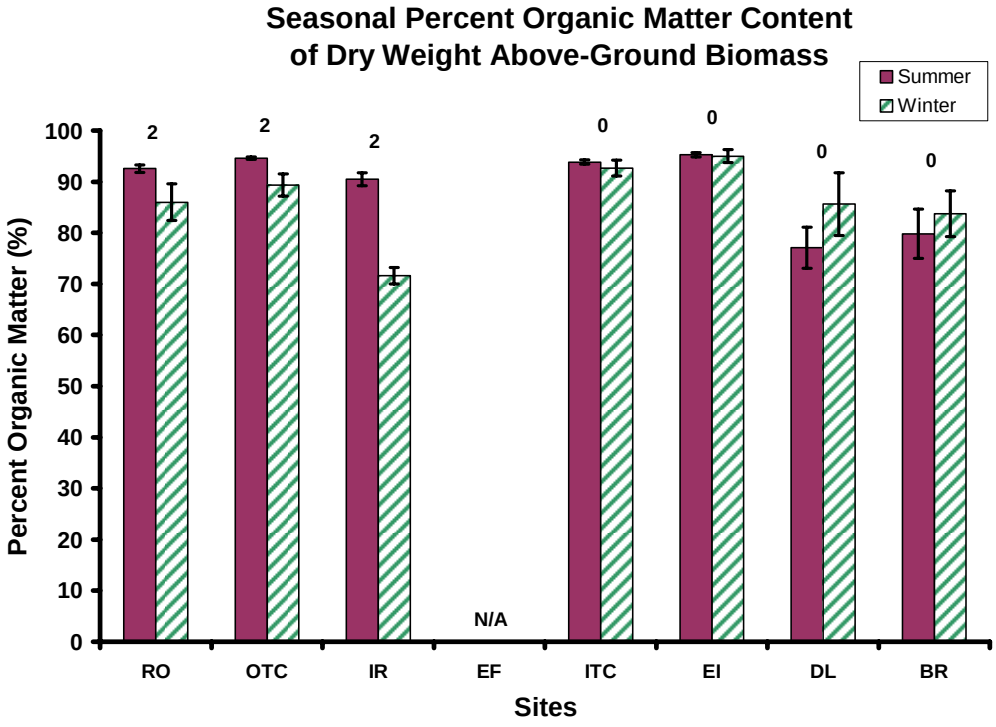
Percent OM content in summer was greater than the percent OM of the sediments in winter for only 3 sites, 3 sites had equal summer and winter OM contents, and 2 sites had winter contents greater than summer (Figure 7a). Percent OM of these sediments did not vary consistently along the general salinity gradient. Significant differences were found for dry weight sediments at sites RO, OTC, DL, and BR for all three seasons ($P > 0.05$), with RO's, OTC's, and DL's contents being greatest in summer and BR's being highest in winter. EF's dry weight sediment organic content was the same between summer and winter. IR's dry weight sediment organic content was also the same for summer and winter, the two seasons in which it was measured ($P \leq 0.05$). No consistent patterns were observed in OM contents for sites in different seasons during the study.

Differences between summer and winter bulk wet measurements of AGB could not be determined since only one sample was taken from each site. However, a consistent pattern was observed in all sites where AGB was present, in which the bulk biomass measurement was always greater in the summer than winter (Figure 8). Differences in percent OM content of the dry weight above-ground biomass were only found in three sites between summer and winter (RO, OTC, and IR) with AGB content being greater in the summer, and the remaining sites showed no difference (Figures 7b and 9). Differences in bulk AGB OM measurement were observed in all sites between summer and winter (Figure 10). The ratios of AGB OM content to sediment OM content ranged from 1.5 in summer RO to almost 4 in winter OTC, showing that the OM content of the AGB was 1.5 to 4 times greater than the sediment OM (Figure 11). Therefore,

Figure 7. Seasonal percent organic matter content. T-tests were performed three times for each site for sediments (spring vs. summer, summer vs. winter, and spring vs. winter) and only one time for each site for above-ground biomass (summer vs. winter) to determine if the percent organic contents were significantly different between seasons. Numbers 1-3 located over a cluster of bars represent a site that was significantly different from season to season ($P > 0.05$). "1" represents a site that was different between spring and summer. "2" represents a site different between summer and winter. "3" represents a site that was different between spring and winter. If a "0" is located over a cluster of bars, no differences were found between seasons ($P \leq 0.05$). Error bars represent one standard deviation. Sediment samples were not collected from IR during the spring, and AGB samples could not be collected from EF since it was a mudflat, so "N/A" (not applicable) is indicated. a.) Percent OM of dry weight sediment is reported. b) Percent OM of dry weight AGB is reported. Note: Scales are different for each graph.


Seasonal Percent Organic Matter Content of Dry Weight Sediment
Percent Organic Matter Content (%)
70
60
50
40
30
20
10
0
Spring
Summer
Winter
1,2,3
1,2,3
N/A
0
1,3
1
2
1,2,3
1,2,3
RO
OTC
IR
EF
ITC
EI
DL
BR
Sites

a.


Seasonal Percent Organic Matter Content of Dry Weight Above-Ground Biomass
Percent Organic Matter (%)
100
90
80
70
60
50
40
30
20
10
0
Summer
Winter
2
2
2
N/A
0
0
0
0
RO
OTC
IR
EF
ITC
EI
DL
BR
Sites

b.

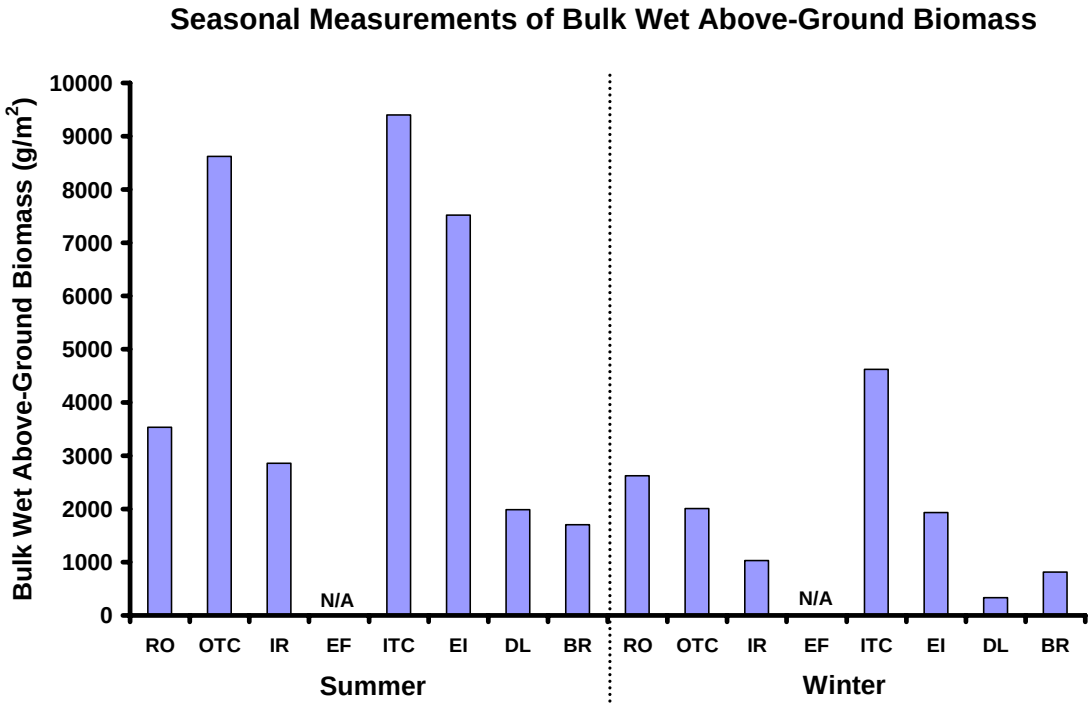


Figure 8. Seasonal measurements of bulk wet above-ground biomass. A "N/A" (not applicable) is reported for EF in both experiments since it was a mudflat and contained no AGB.

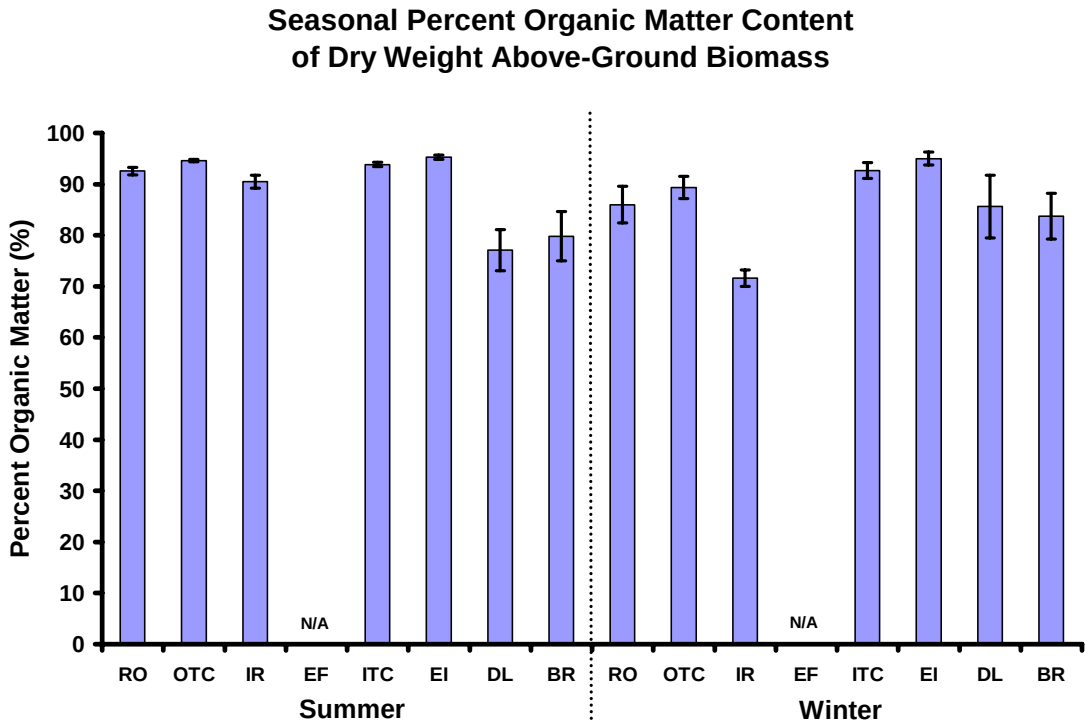


Figure 9. Seasonal percent organic matter content of dry weight above-ground biomass. Error bars represent one standard deviation. A "N/A" (not applicable) was reported for EF in both graphs since it was a mudflat and contained no AGB.

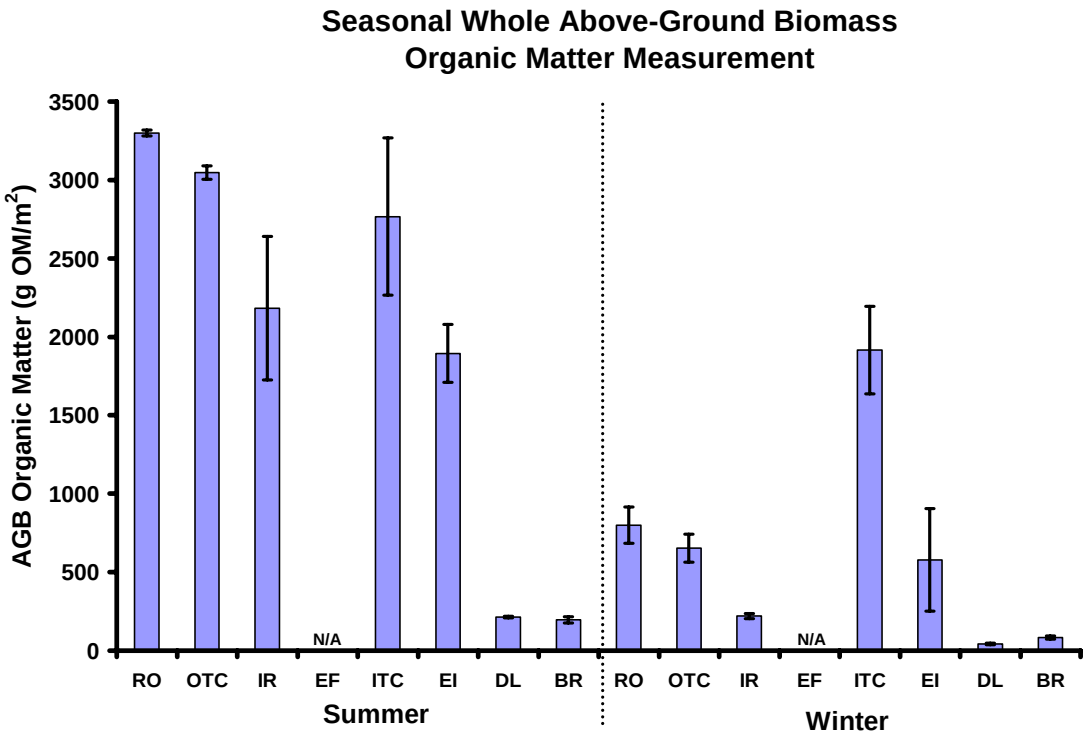


Figure 10. Seasonal whole above-ground biomass organic matter measurement. Error bars represent one standard deviation. A "N/A" (not applicable) was reported for EF in both experiments since it was a mudflat and contained no AGB.

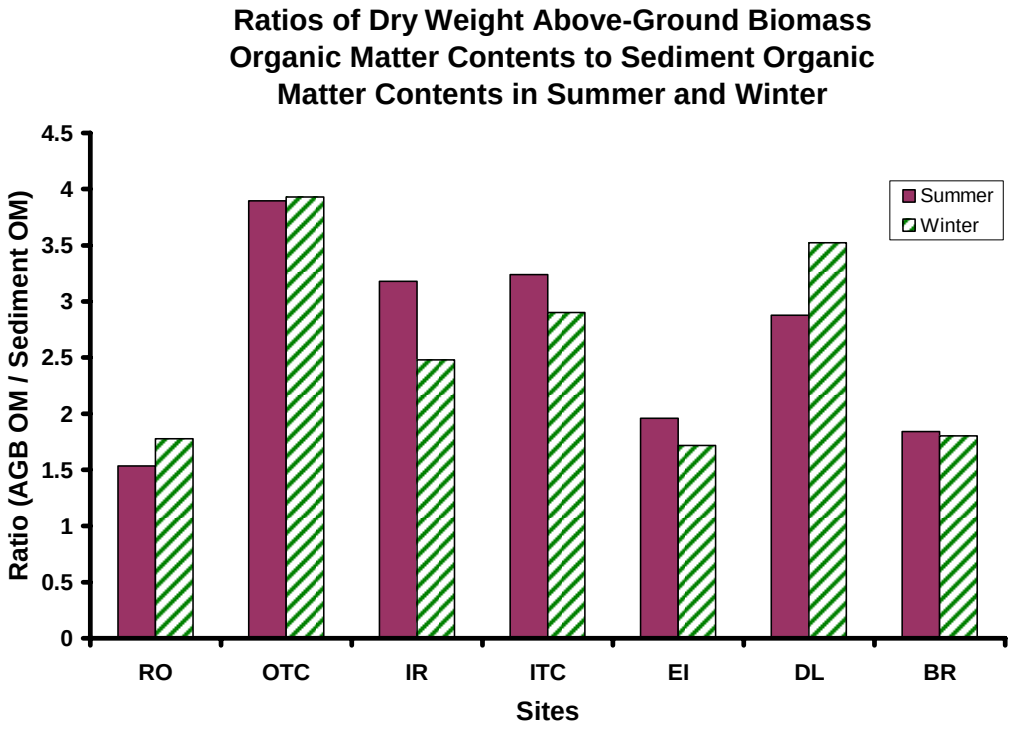


Figure 11. Ratios of dry weight above-ground biomass organic matter contents to sediment organic matter contents in summer and winter. The dry weight OM content of the AGB was divided by the dry weight OM to determine whether the AGB OM contents were reflected in the sediment OM contents.

there was no direct reflection of AGB OM in the sediment OM. Also, there was no pattern observed seasonally or along a general salinity gradient.

DISCUSSION

Rates of Methane Production and Sulfate Reduction

The methane production and sulfate reduction rates found in this study exhibit a wide range, and encompass ranges reported in the literature (Table 2). Control samples were used for comparison of MP and SR rates to literature values because they best reflect *in situ* processes. It should be noted that samples from each season were actually stored at the same laboratory temperature during incubation in order to eliminate temperature as a variable in this study, which was designed to determine the effects of sulfate supply on remineralization of organic matter. Therefore, winter rates reported in this study represent potential remineralization rates at 23ºC, which may be higher than actual *in situ* temperatures depending on short-term temperature variations. Rates of MP and CDP during the winter likely overestimate what would actually be occurring *in situ*. Table 2 lists a wide variety of sediment types and their corresponding MP and SR rates.

The control MP rates measured in this study were generally greater than those reported in the literature for marine and saltmarsh sediments, displaying the impact that salinity has on inhibiting methanogenesis. Control SR rates in this study were similar to most of the SR rates indicated in the literature for the different types of sites. The span of rates from the current study actually expand the range of reported rates for similar types of sediments, with rates from sites in the current study being both greater than and less than reported literature ranges, indicating that the sites in this study encompass a wide variety of estuarine sites with diverse geochemical settings.

Table 2. Rates of methane production and sulfate reduction in different types of sediments. Rates were measured by various techniques including flux measurements, tracers, and slurries. They were converted to μM/hr where necessary and are reported (sometimes as ranges) in μM/hr. An assumed bulk density of 1.5 g/cm^3 was used where needed to convert measurements from area flux measurements to volumetric measurements (Capone and Kiene 1988). SR rates are given for non-altered sediments unless followed by an "(A)", which indicates the sample was amended with a sulfate solution. Methods were not always indicated for rates that were referenced in other sources. A "--" indicates where no rate was reported.

Site	Methane Production	Sulfate Reduction	Reference
Marine and Saltmarsh			
Cape Lookout Bight, NC	3.29	12.5	Hoehler et al. 1999
Cape Lookout Bight, NC	0.06-5.7	2.74-12.6	Crill and Martens 1983
Cape Lookout Bight, NC	12.7	--	Crill and Martens 1982[a]
Cape Lookout Bight, NC	--	83.8	Klump 1980, Bartlett 1981, and Crill and Martens 1983 [a]
Saanich Inlet, BC, Canada	--	2.05-12.0	Devol et al. 1984
Limfjorden, Denmark	--	1-8.3	Jørgensen 1977[b]
Sapelo Island, GA, *Spartina*	0.022-11	--	King and Wiebe 1978 [b]
Sapelo Island	--	7.38-23.9	Skyring et al. 1979[c]
Sapelo Island	--	45.8-52.4	King 1983 [c]
Sapelo Island	--	6.25-12.5	Howarth and Giblin 1983 [c]
Sapelo Island	--	11.7-23.8	Howarth and Merkel 1984[c]
Sapelo Island	--	13.8-25.0	Howes et al. 1984 [c]
Long Island Sound, Saltmarsh	--	0.75-10.0	Goldhaber et al. 1977[c]
Delaware Inlet, NZ	0.25-1.8	0.3-2.9	Mountford et al. 1980 [b]
Lowes Cove, Mud	0.4-1.4	<4.2- >42	King et al. 1983 [b]
Brittany Mudflat	0.16	115	Winfrey and Ward 1983 [b]
Sippewissett Saltmarsh	0.43	10.4-250	Howarth and Teal 1979 [b]
Sippewissett Saltmarsh	--	4.63-102	Howarth and Merkel 1984[c]
Sippewissett Saltmarsh	--	23.3-44.6	King et al. 1985 [c]

Great Sippewissett Saltmarsh, MA	--	2.67-14.1	Hines et al. 1994
Louisiana Saltmarsh	8.97	9.13 (A)	D'Angelo and Reddy 1999
Saltmarsh Drainage Creek, UK	0.0003-0.0012	0.17-1.17	Senior et al. 1982
Saltmarsh Pan, UK	0.0005-0.0011	0.083-1.67	Senior et al. 1982
Saline Marsh, Coastal LA	2.60-7.81	--	Crozier and DeLaune 1996

Estuarine			
Cape Fear River Estuary, NC (Intertidal)	1.30-19.0	1.42-38.5	**This study**
Cape Fear River Estuary, NC (Intertidal)	2.19-12.0	5.67-20.7 (A)	Sexton 2002
Intermediate Marsh, Coastal LA	28.6-32.6	--	Crozier and DeLaune 1996
Brackish Marsh, Coastal LA	7.81-19.6	--	Crozier and DeLaune 1996
Kingoodie Bay, UK (Intertidal estuarine mudflat)	0.018	3.01	Wellsbury et al. 1996
Aust Warth, UK (Dynamic estuary)	0.021	1.31	Wellsbury et al. 1996
Long Island Sound, Estuarine Sediment	--	0.2-11.8	Goldhaber et al. 1977[c]
Long Island Sound, Estuarine Sediment	--	3.31-4.45	Aller and Yingst 1980[c]
Long Island Sound, Estuarine Sediment	--	1.14-2.51	Bordeau and Westrich 1984[c]
Long Island Sound, Estuarine Sediment	--	8.9-40.1	Berner and Westrich 1984[c]
Great Bay Estuary, NH	--	1.04-10.4	Hines et al 1982[c]
Great Bay Estuary, NH	--	175.8	Nakai and Jensen 1964[c]
Great Bay Estuary, NH	--	1.25-14.6	Hines and Jones 1985

Freshwater and Peatland			
Lower Cape Fear River Basin, NC (FW, Submerged)	7.83-44.0	15.2-61.6 (A)	Sexton 2002
Fresh Marsh, Coastal LA	19.6	--	Crozier and DeLaune 1996
Ashleworth Quay, UK, Riverside Mudbank	3.47	0.438	Wellsbury et al. 1996
Bleak Lake Bog, Canada	0.079	1.67	Vile et al. 2003

Červené Blato and Oceán Bog, Czech Republic	0.008-0.017	31.7-33.3	Vile et al. 2003
Houghton Lake Peat, MI	10.3	8.60 (A)	D'Angelo and Reddy 1999
Everglades, FL, Peat	3.80-4.11	6.04-6.29 (A)	D'Angelo and Reddy 1999
Ellergower, Scotland, Hollow Peat	1.0-6.5	2.24-16.8 (A)	Watson and Nedwell 1998
Ellergower, Scotland, Hummock Peat	--	1.15-3.49 (A)	Watson and Nedwell 1998
Great Dun Fell, England, "Wet" Peat	0.25-0.425	0.181-9.93 (A)	Watson and Nedwell 1998
Great Dun Fell, England, "Dry" Peat	--	0.139-0.833 (A)	Watson and Nedwell 1998
Talladega, AL, Mineral	6.44	5.35 (A)	D'Angelo and Reddy 1999
Parnell Prairie Pothole, ND, Mineral	10.9	12.0 (A)	D'Angelo and Reddy 1999
Crowley Paddy Soil-Silt, LA	3.13	3.13 (A)	D'Angelo and Reddy 1999
Lake Mendota, WI	1.21	0.29	Ingvorsen and Brock 1982
Lake Washington, WA	0.021-0.17	0.072	Kuivila et al. 1989
Wintergreen Lake A	40.0	6.20	Lovley and Klug 1982, Lovley et al 1982, Smith and Klug 1981, and unpublished data[d]
Wintergreen Lake B	26.0	4.0	Lovley and Klug 1982, Lovley et al 1982, Smith and Klug 1981, and unpublished data[d]
Lawrence Lake	1.8	4.6	Lovley and Klug 1983[d]
Lake Vechten	34.0	--	Cappenberg 1974[d]

Other			
Belhaven, NC, Muck	2.99	6.53 (A)	D'Angelo and Reddy 1999
Florida, Muck	6.10	2.44 (A)	D'Angelo and Reddy 1999
Michigan Peat, Impacted by domestic waste	20.5	36.5 (A)	D'Angelo and Reddy 1999

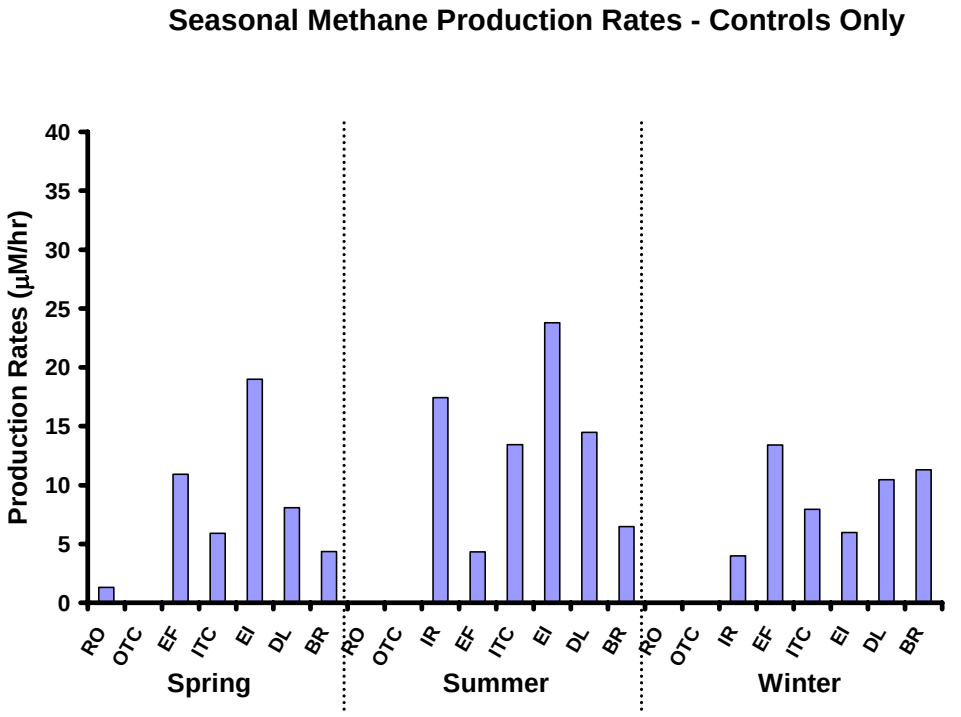
[a] Reference from Martens and Klump 1984
[b] Reference from Capone and Kiene 1988
[c] Reference from Skyring 1987
[d] Reference from Lovley and Klug 1986

Salinities and Sulfate Concentrations of Sites

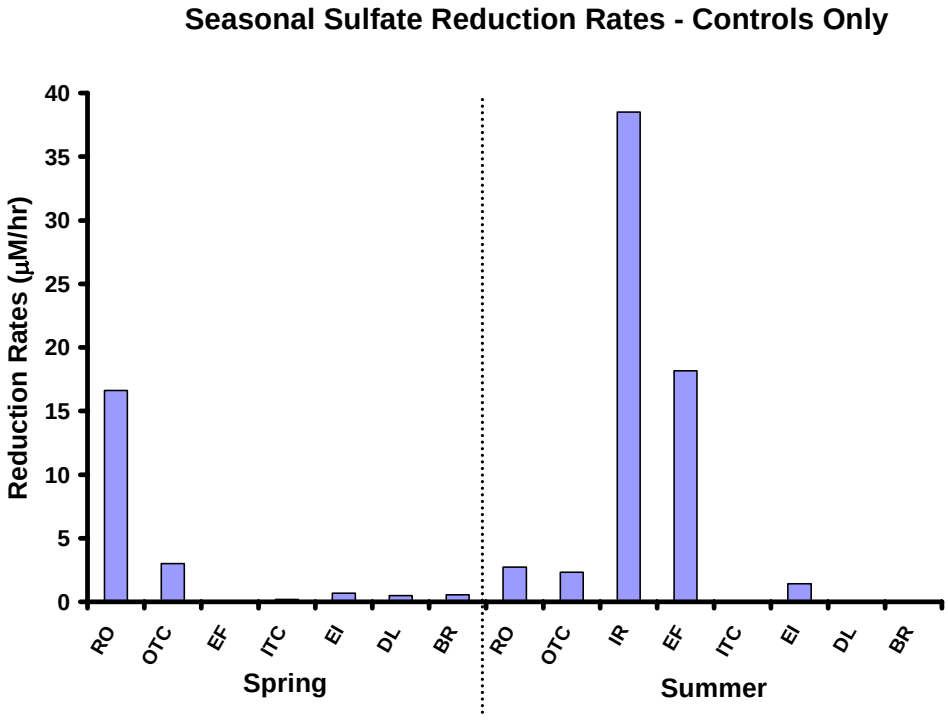
The salinities and sulfate concentrations of porewaters at the sites in this study generally controlled the type of remineralization processes that took place at those sites. Control methanogenic rates were generally greater during the summer than the spring and winter (low SO_4^{2-}) (Figure 12a). Control sulfate reduction rates were greater for some sites in the summer than spring and greater for the other sites in spring than summer (Figure 12b). Control carbon dioxide production rates were generally greater in summer than spring (5 out of 7), and winter rates were generally greater than summer (6 out of 8) (Figure 12c). The increase in CDP in winter was due to more SR in the winter from increased salinities and not increased OM or bioavailability of OM.

Higher sulfate concentrations resulted in relatively more SR (Figure 4), and lower sulfate concentrations allowed more MP to occur (Figure 3). Cape Fear River salinities typically vary temporally and are higher in the summer than in the winter. In the current study, however, salinities (and thus sulfate concentrations) were slightly greater in the winter, although both seasons were fresher than the baseline data (Figures 13 and 14). The salinities and sulfate concentrations of sites in this study did vary by season, but they were opposite of what is normally observed.

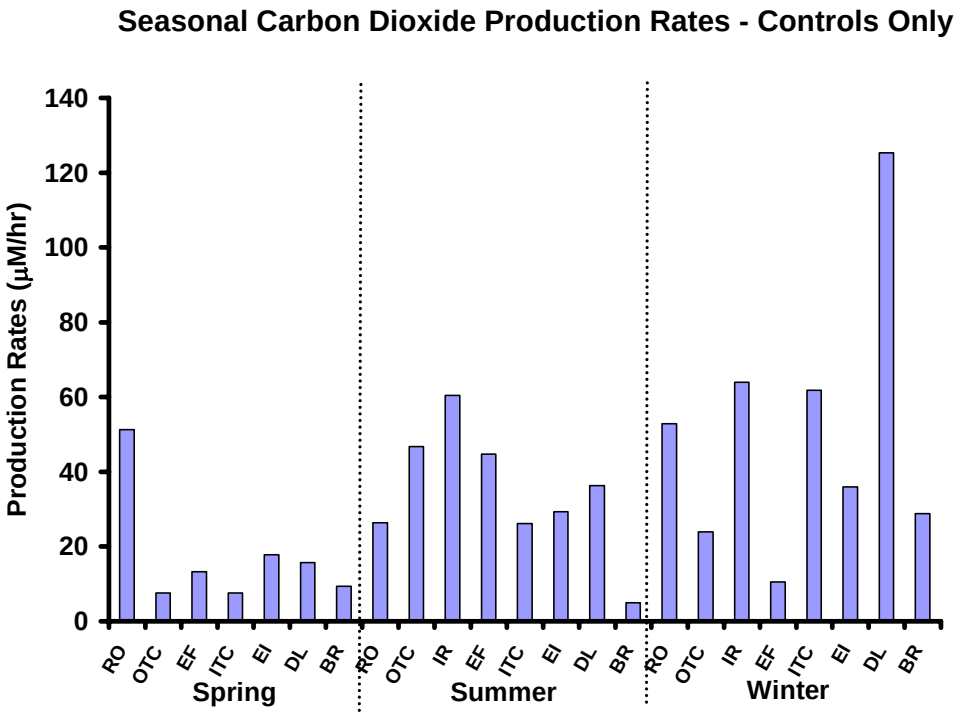
During the current study period, sites were very fresh in both the summer (0.1-0.6 ppt for EI) and winter (0.1-1.9 ppt for EI); however, winter had slightly higher salinities than summer. The fact that summer was slightly less saline compared to winter was due to high streamflows experienced during the study. Streamflows were greater than normal in the summer and lower than normal in the winter. Figure 13 provides salinity data from Eagle Island (EI), a representative site in both the current study and the


Seasonal Methane Production Rates - Controls Only
Production Rates (µM/hr)
0
5
10
15
20
25
30
35
40
RO
OTC
EF
ITC
EI
DL
BR
RO
OTC
IR
EF
ITC
EI
DL
BR
RO
OTC
IR
EF
ITC
EI
DL
BR
Spring
Summer
Winter

a.


Seasonal Sulfate Reduction Rates - Controls Only
Reduction Rates (µM/hr)
0
5
10
15
20
25
30
35
40
RO
OTC
EF
ITC
EI
DL
BR
RO
OTC
IR
EF
ITC
EI
DL
BR
Spring
Summer

b.



c.

Figure 12. Seasonal methane production, sulfate reduction, and carbon dioxide production rates for controls. Note: Scale is different on Graph c. This figure presents the data from Figures 3 through 5 in another way.

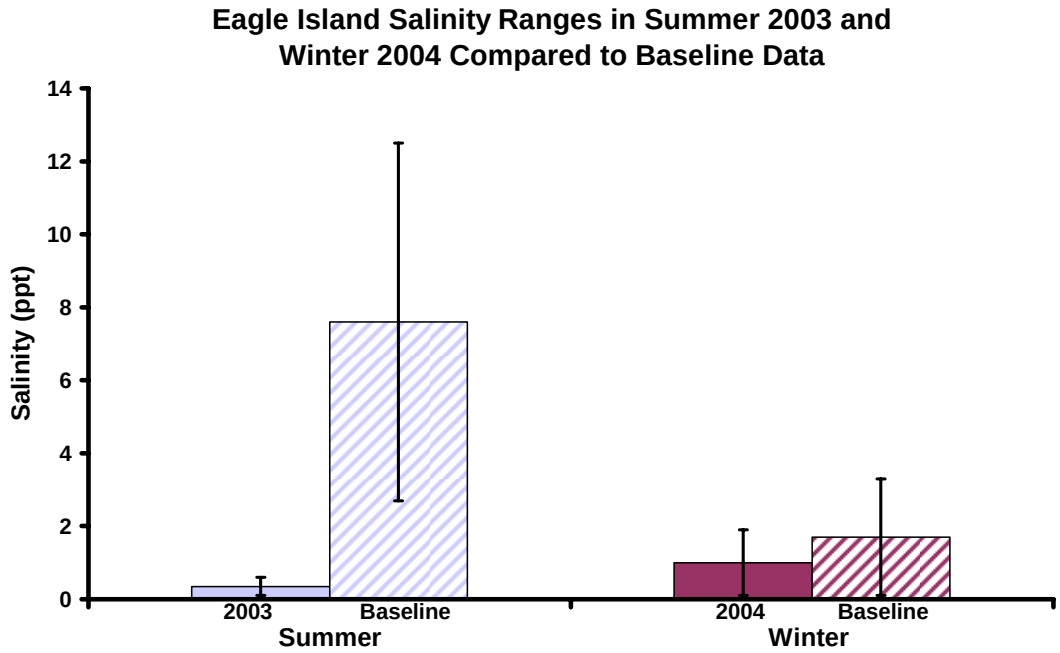


Figure 13. Eagle Island salinity ranges in summer 2003 and winter 2004 compared to baseline data. Baseline data were compiled from August 1, 2000, to May 31, 2003, from the Eagle Island monitoring station located closest to the river bank. Salinity data were obtained from the Army Corp of Engineers Cape Fear River Monitoring Project (CFMP). Errors bars represent the ranges of salinities obtained for Eagle Island (EI). Solid columns are EI data obtained from the most recent CFMP (Hackney et al. 2005). Striped columns are EI data considered baseline data for each season (Hackney et al. 2002, 2003, 2004). Refer to Figure 1 for the location of EI on the Cape Fear River.

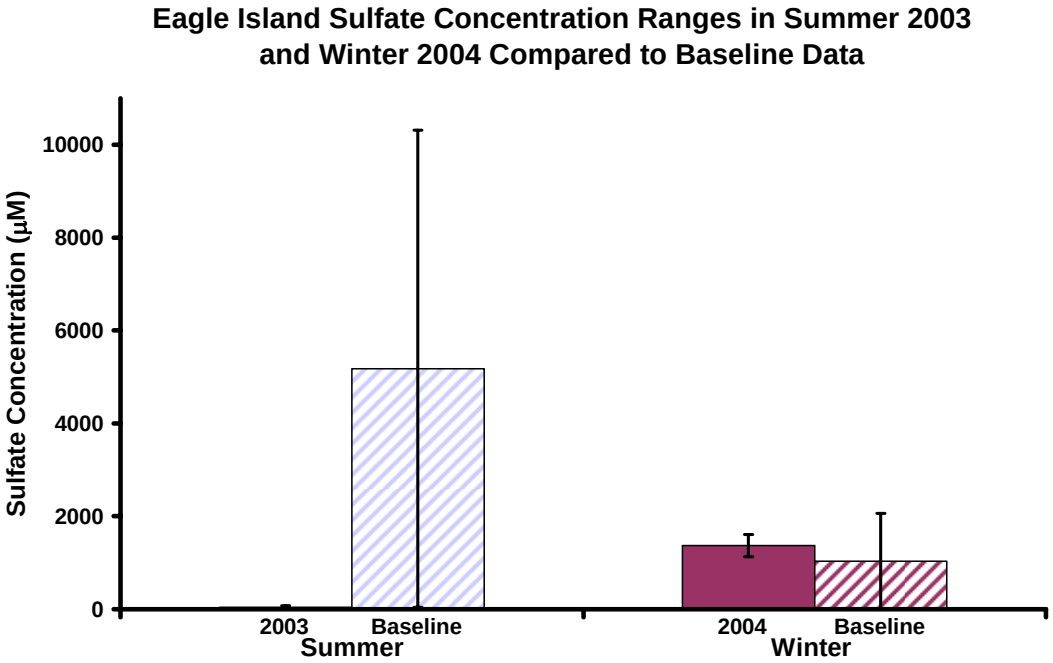


Figure 14. Eagle Island sulfate concentration ranges in summer 2003 and winter 2004 compared to baseline data. Baseline data were compiled from August 1, 2000, to May 31, 2003, from the Eagle Island monitoring station located closest to the river bank. Sulfate data were obtained from the Army Corp of Engineers Cape Fear River Monitoring Project (CFMP). Errors bars represent the ranges of sulfate concentrations obtained for EI. Solid columns are EI data obtained from the most recent CFMP (Hackney et al. 2005). Striped columns are EI data considered baseline data for each season (Hackney et al. 2002, 2003, 2004).

CFMP, that has an intermediate salinity regime and location on the river compared to the other sites. Salinity data from EI during summer 2003 and winter 2004 (current project dates) were compared to data from August 2000 to May 2003 (first years of CFMP in which EI salinity baselines were established).

Normal conditions for sulfate concentrations during the study would have included higher concentrations during the summer and lower concentrations during the winter. Baseline sulfate concentrations during summer for the representative site EI in the CFMP range from 39 μM to 10 x 10^3 μM, whereas baseline winter sulfate concentrations for EI range from 4 μM to 2 x 10^3 μM (Hackney et al. 2002, 2003, 2004). During the current study period, sites had low sulfate concentrations in both the summer 2003 and winter 2004 compared to the baseline data. Winter actually had higher sulfate concentrations than summer, which is also opposite of the baseline conditions. Figure 14 provides sulfate data from EI in the CFMP for summer 2003 and winter 2004 and compares the data to EI's baseline sulfate concentration data.

Normal Seasonal Salinity Variations

The amount of sulfate available in a site depends on two factors: microbial consumption via SR and input from salinity (Crill and Martens 1983). Generally, during the summer there may be a larger amount of sulfate input to the estuary because seawater carrying sulfate can intrude further upstream during times of traditionally lower river flow and higher evaporation (USGS 2004). However, activity of sulfate-reducing bacteria, and thus consumption by the bacteria, is greater because of increased ambient temperatures (Hines et al. 1989). The consumption processes override the input resulting

in relatively low sulfate concentrations compared to the high salinity inputs. However, these sulfate concentrations are still higher than normal winter sulfate concentrations (Hackney et al. 2002, 2003, 2004).

Generally, during winter, the opposite of the summer process occurs. There may be less sulfate input to the estuary due to higher river flow rates (USGS 2004). Also, consumption by sulfate-reducing bacteria is lower because of decreased ambient temperatures (Hines et al. 1989). The net result of these competing processes in the winter is that the small amount of sulfate that does make it into the system is consumed over time, keeping concentrations low (Hackney et al. 2002, 2003, 2004).

Cape Fear River Streamflows During the Study Period

Floodwater is typically more saline during the summer than winter, which can be attributed to streamflows. Streamflows for the lower Cape Fear River are generally higher in the winter and lower during the summer (USGS 2004). Baseline salinities for sites in the CFMP during summer range from less than 1 to approximately 13 ppt, whereas baseline winter salinities range from less than 0.1 to approximately 0.3 ppt (Hackney et al. 2002, 2003, 2004).

The seasonal salinity and sulfate concentration patterns observed during the study period reflect the monthly streamflow statistics for the Cape Fear River (USGS 2004, Hackney et al. 2005). During May and June of 2003, when spring samples were collected, the monthly mean streamflows of the Cape Fear River were 227.7 m^3/s and 309.2 m^3/s, respectively. These monthly means are at least twice the means averaged over 33 years since 1970, which are 133.7 m^3/s for May and 109.9 m^3/s for June (USGS

2004). During August and September of 2003, when summer samples were collected, the monthly mean streamflows were 379.7 and 191.3 m^3/s, respectively. These means are two to four times greater than the means averaged over 34 years since 1969, which are 93.1 m^3/s for August and 101.4 m^3/s for September. Therefore, in summer there was a greater amount of streamflow in the Cape Fear than normal, which reduced salinities and sulfate concentrations at the study sites.

During January and February of 2004, when winter samples were collected, the monthly mean streamflows were 87.8 m^3/s and 214.6 m^3/s, respectively. These means are one-third and three-fourths the means averaged over 33 years since 1970, 242.0 m^3/s and 278.3 m^3/s for January and February, respectively. Therefore, winter streamflow was much lower than normal, and higher-salinity water supplying higher sulfate concentrations penetrated further upstream. The combination of these abnormal seasonal streamflows resulted in an opposite pattern of seasonal salinities and sulfate concentration variations.

Effects of Increased Sulfate Concentrations on Rates of Remineralization

Previous research has shown that freshwater and sulfate-depleted brackish water samples with sulfate amendments have greater carbon remineralization rates than controls (Sexton 2002). However, this process has not been examined in estuarine samples containing higher levels of salt and experiencing wider ranges of salinities and sulfate concentrations. In general, the net effects of sulfate additions on remineralization rates depended on the increases in CO_2 production via increased SR versus the amount of inhibition of remineralization via decreases in MP.

Effect of Artificial Sulfate Additions

In the current study, amended samples generally had greater remineralization rates than controls, meaning that the addition of sulfate, which would occur with greater salinities, increased remineralization rates (Figures 4 and 5). During the summer when sulfate concentrations were relatively lower and sulfate was limited, more than half of the sites (5 out of 8) responded with greater CO_2 production rates for amended samples versus controls (Figure 5b), meaning that sulfate concentrations had been below the threshold concentration for SR in control samples. The sulfate concentrations of the samples in this study during the summer ranged from 83 μM to 11 x 10^3 μM. Based on Sexton's (2002) sulfate threshold concentration of 92 μM, some of the sites in the current study had sulfate concentrations below this value, while others were elevated, once again showing that estuarine samples may have a different threshold value. In general, these results agree with Sexton's. When values were below the threshold value, less SR occurred. These results suggest that artificial sulfate additions can have a great impact on CDP when sulfate is initially limited in a sample.

Methane production rates were inhibited by increased sulfate concentrations from artificial sulfate additions in summer, as all samples that produced methane (6 out of 6) had greater MP rates in control samples than amended samples (Figure 3b). When sulfate concentrations were slightly greater, such as in winter (based on the CFMP EI data), the sediments also had a response to artificial sulfate additions. MP rates during winter in control samples were equal to or greater than amended MP rates in almost all of the sites in which methane was produced (5 out of 6). Therefore, low sulfate

concentrations during the summer and winter resulted in increased inhibition of methanogenesis with the addition of sulfate.

The addition of sulfate to samples has two effects on remineralization rates, e.g. it inhibits MP and stimulates SR. The effect of sulfate additions on net remineralization rates between winter and summer (Figure 15) were calculated by adding the net carbon produced by MP in samples (amended MP minus control MP) to the net carbon produced by CO_2 production via SR in samples (amended CDP minus control CDP). Sites with positive data indicate that carbon dioxide production had been stimulated by increases in sulfate more than methane production had been inhibited. Sites with negative data indicate that methane production had been inhibited by increases in sulfate concentrations more than carbon dioxide production had been stimulated.

During this study, 4 sites in summer and 6 in winter responded in the same way to artificial sulfate additions as Sexton's 2002 study. They reacted similarly likely because Sexton's samples had been sulfate-depleted prior to the sulfate additions, just like many of the sites in the current study, due to the study year being one of the freshest on record. Therefore, if a site is depleted in sulfate, as these sites were during summer and winter, then a sulfate concentration increase can cause increases in net remineralization.

Effect of Seasonal Sulfate Variations on Remineralization Rates

Winter sulfate concentrations were slightly elevated compared to the summer. The seasonal effects of *in situ* sulfate variations can be seen by the net remineralization rates (Figure 16), which were calculated by adding the decrease in MP during winter (winter MP minus summer MP) to the increase in CO_2 production via SR in the winter

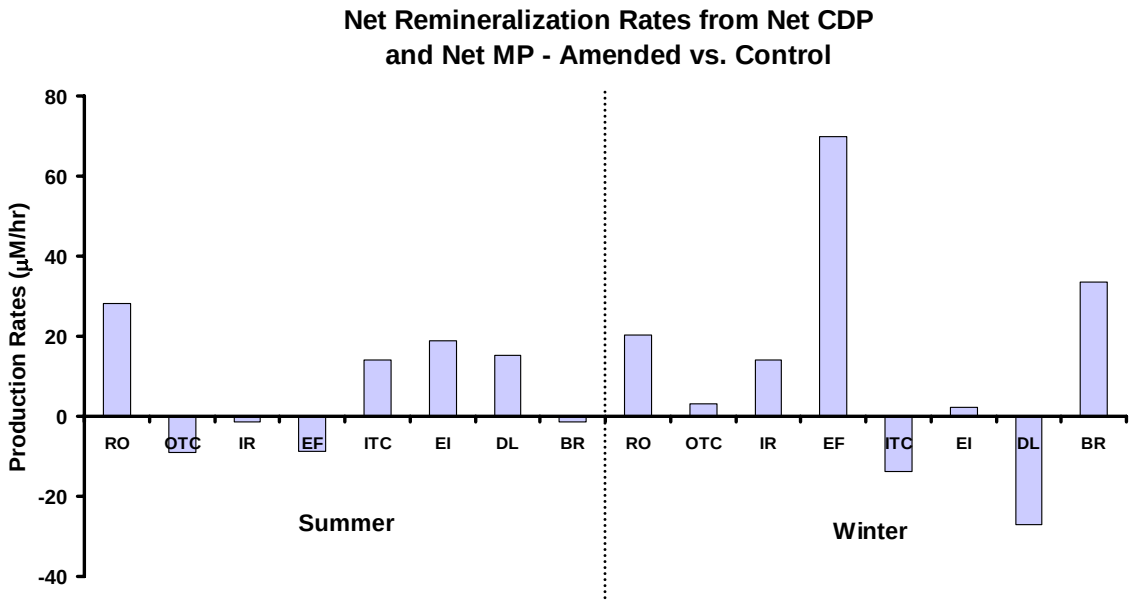


Figure 15. Net remineralization rates from CDP stimulation and MP inhibition in amended vs. control samples. The effects of sulfate addition can be seen by the net remineralization rates, which were calculated by adding the net carbon produced by MP in samples (amended MP minus control) to the net carbon produced by CO_2 production via SR in samples (amended CDP minus control CDP). Sites with positive data indicate that carbon dioxide production had been stimulated by increases in sulfate more than methane production had been inhibited. Sites with negative data indicate that methane production had been inhibited by increases in sulfate more than carbon dioxide had been stimulated.

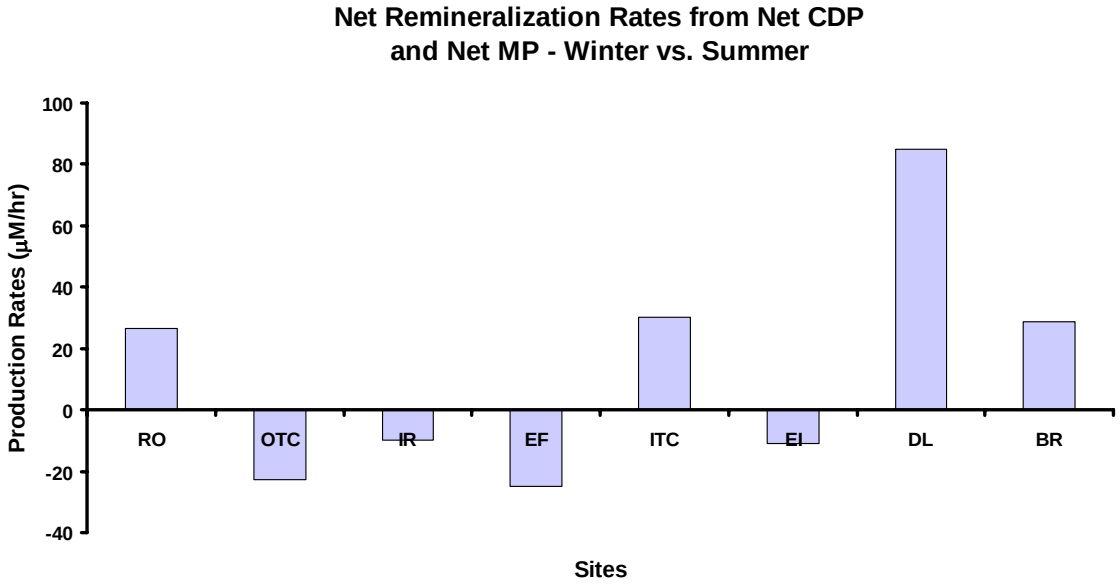


Figure 16. Net remineralization rates from net CDP and net MP – winter vs. summer. The effects of sulfate increases can be seen by the net remineralization rates, which were calculated by adding the decrease in MP during winter (winter MP minus summer) to the increase in CO_2 production via SR in the winter (winter CDP minus summer CDP). Sites with positive data indicate that carbon dioxide production had been stimulated by increases in sulfate more than methane production had been inhibited. Sites with negative data indicate that methane production had been inhibited by increases in sulfate more than carbon dioxide had been stimulated.

(winter CDP minus summer CDP). Sites with positive data indicate that carbon dioxide production had been stimulated by increases in sulfate more than methane production had been inhibited. Sites with negative data indicate that methane production had been inhibited by increases in sulfate more than carbon dioxide had been stimulated. There were slight seasonal variations in initial sulfate concentrations of samples, but there was no consistent effect on net remineralization. In half of the sites, higher seasonal sulfate concentrations in winter resulted in higher net remineralization, while the other half had lower remineralization rates with higher sulfate concentrations. The lack of response is likely due to the lack of significant variations in sulfate concentrations between these two seasons. During a normal year, seasonal variations in sulfate concentrations may have a more important impact on remineralization rates.

Effect of Seasonal Sulfate Variations on Total Carbon Remineralized

The total amount of carbon remineralized in control samples during summer and winter is another measure of seasonal sulfate variations on net remineralization. Unlike the comparison described above, TCR represents a longer term measurement than the initial rates observed during the incubation experiments. Most of the sites (6 out of 8) had greater TCR in the winter than summer (Figure 6) likely due to the higher sulfate concentrations during winter and longer SR times. It was not attributable to OM content since the OM content actually decreased in some sites, while the remineralization rates increased.

The results described above are sometimes in contrast to the current understanding of carbon remineralization in organic rich sediments. Changes in

respiration rates generally have been thought to be controlled mainly by organic matter quantity and quality when the necessary oxidant is present in adequate concentrations (Henrichs and Reeburgh 1987, Capone and Kiene 1988, Watson and Nedwell 1998, Vile et al. 2004). One might argue that winter remineralization rates were greater than summer rates because there may have been a large amount of fresh labile matter available for decomposition during winter. It has been observed that anaerobic processes can be enhanced by the addition of OM (Andersen and Hargrave 1984). However, the argument does not apply in all cases in this study because percent OM contents of the sediments in three sites (RO, OTC, and DL) were greater during the summer (Figure 7a), and net remineralization rates of two of them (RO and DL) increased in the winter (Figure 16). Also, the percent OM content was the same in three sites (IR, EF, and ITC) from summer to winter (Figure 7a) and the net remineralization rate of at least one (ITC) was greater in the winter (IR and EF had lower rates in winter) (Figure 16). Therefore, the driving force in the current study generally was not OM but was instead sulfate.

Remineralization Processes

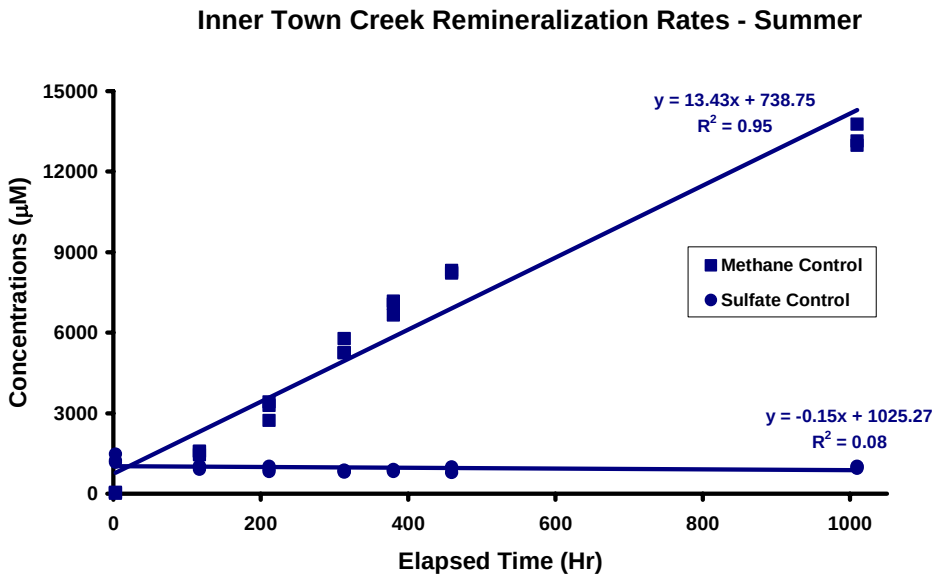
MP and SR are terminal electron acceptors in a long line of thermodynamically favorable electron acceptors for respiration. The aerobic and anaerobic microbial communities use these electron acceptors in the order O_2, NO_3^{2-}, MNO_2, Fe(III), SO_4^{2-}, and CO_2 (Capone and Kiene 1988, D'Angelo and Reddy 1999). Therefore, when the other electron acceptors are depleted and only SO_4^{2-} is present in adequate concentrations, respiration will occur via sulfate reduction. However, when neither SO_4^{2-} nor the other electron acceptors are present, respiration will proceed by fermentation or

CO_2 reduction (also called MP). This explains why saline marshes (containing high levels of SO_4^{2-}) most commonly undergo SR, and freshwater swamps and lakes (containing low levels of SO_4^{2-}) will have more MP (Sexton 2002).

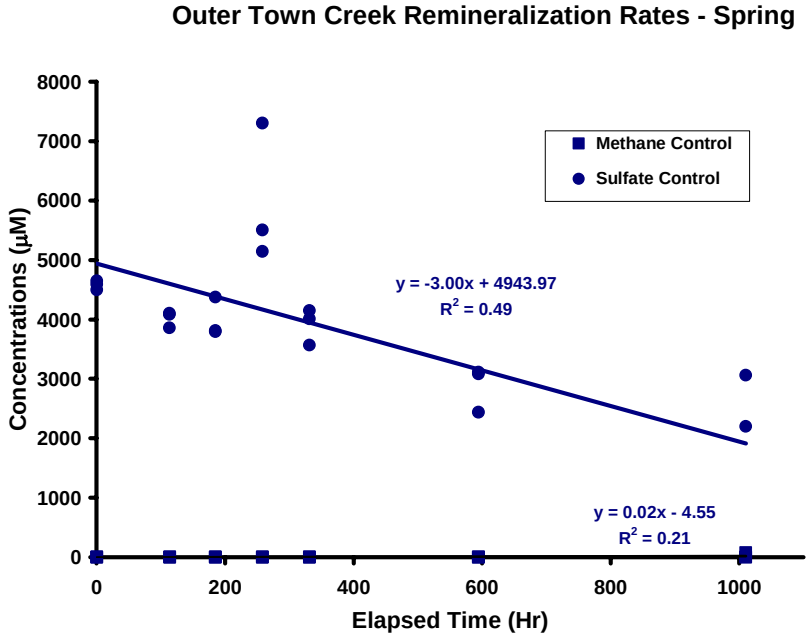
Sulfate reduction did not occur in some sites during the study. In order for SR to occur, a minimum concentration of sulfate is needed. In samples where MP was present and no SR was observed, concentrations of sulfate were too low for SR to proceed and also too low to inhibit MP. This result was observed in summer experiments for ITC, DL, and BR; and during spring for EF. For example, when the concentration of sulfate was 1244 μM during summer in the control ITC sample, methane production was not inhibited (Figure 17a). This sulfate concentration is significantly higher than the sulfate-reducing threshold concentration determined by Sexton (2002) of 92 μM and shows that estuarine samples may have a different threshold value compared to fresher sites.

Methane production also did not occur in some sites during the study. Generally, sulfate reducers out-compete methanogens for substrates. Therefore, in samples where SR was present and no MP was observed, methane producers were competitively inhibited by sulfate reducers. This result was observed during the spring experiments for OTC (Figure 17b). During the spring experiment at OTC, when the initial OTC control sulfate concentration was 4580 μM, well above the threshold concentration of 92 μM (Sexton 2002), methane was not produced.

In one site neither MP nor SR were observed, even though carbon dioxide was produced. CDP without MP and/or SR was in contrast to the rest of the experiments in which MP and/or SR were responsible for the production of carbon dioxide. Since carbon dioxide was produced in these samples, an external oxidant must have been



a.



b.

Figure 17. Inner Town Creek and Outer Town Creek remineralization rates during summer and spring, respectively. Rates are indicated by the slopes of the lines. R-squared values are given to show strength of correlation. Significance was determined. a.) Control MP and SR rates are shown for ITC in summer. The MP rate is significant ($P \leq 0.05$), but the SR rate is not ($P > 0.05$). b.) Control MP and SR rates are shown for OTC in spring. The SR rate is significant ($P \leq 0.05$), but the MP rate is not ($P > 0.05$). Note: Scales are different for each graph.

present that pre-empted SR and MP. The oxidant present in the OTC samples that had CDP but no MP or SR might have been iron (III). A buildup of an orange-colored ring at the air/water interface was observed in stored centrifuge tubes in which the samples were centrifuged for IC analysis. It is possible that the orange-colored ring was precipitated iron. Iron reduction would have inhibited any SR or MP from occurring until iron was depleted. Lovley and Phillips (1987) and van Bodegom et al. (2004) illustrated this point by inhibiting SR and/or MP with the addition of ferric iron to sediments dominated by those processes. An orange-colored ring was also observed in stored IR centrifuge tubes, but IR's MP and SR were not inhibited.

This result may have important implications in that studies looking at only MP and SR may be missing processes that are important to the overall remineralization of OM. Futhermore, surface processes occurring in the top few millimeters of sediments are often not measured via traditional geochemical methods. Microbial remineralization processes involving oxygen, iron, and manganese may be overlooked due to their occurrence at the top few millimeters of the sediment surface and the difficulty in measuring these redox-sensitive species without causing changes in concentrations due to oxidation. These processes may be very important to the overall remineralization of organic matter since the freshest and most labile material is most likely at the surface of the sediments where it is deposited.

Coexistence of Methane Production and Sulfate Reduction

During the course of research, an unanticipated microbial process was discovered that showed for the first time that MP can occur at the same time and at approximately

the same rate as SR in wetland sediments. When MP and SR occur simultaneously in the same soil profile, they are usually separated spatially by biogeochemical zonation (Lovley and Klug 1986, Kuivila et al. 1989) because of competitive inhibition. Sulfate-reducing bacteria will usually out-compete methane-producing bacteria, thus inhibiting MP (King and Wiebe 1980, DeLaune et al. 1983, Watson and Nedwell 1998, Dise and Verry 2001, Gauci et al. 2002). During the current study however, MP and SR occurred at the same time throughout two of the experiments at rates of comparable magnitudes, e.g. SR to MP final rate ratio of 2 to 1 for IR Control in summer (Figure 18) and 1 to 1 for OTC Control in summer. (IR and OTC were two of the three most saline sites.)

The idea of coexistence of MP and SR is not a new one. It has been observed by other researchers in the past. However, MP and SR have not been observed before to coexist with rates of such comparable magnitudes to each other as they did in this study. MP has sometimes been observed during SR, but usually only in trace amounts.

Senior et al. (1982) observed measurable rates of MP in sulfate-reducing sediments, but the SR rates were about 3 orders of magnitude greater than MP rates. Crill and Martens (1983) observed the simultaneous occurrence of MP and SR that may have involved organisms that can produce methanogenic substrates. In their study the SR rates were one order of magnitude greater than MP rates. Capone and Kiene (1988) observed low but measurable rates of MP occurring in sulfate-reducing sediments, but the rates of SR were generally 1 to 3 orders of magnitude greater than MP. Vile et al. (2003) reported that MP and SR co-occurred in peatlands along a sulfur-deposition gradient where SR was generally 3 orders of magnitude greater than MP. Watson and Nedwell (1998) measured rates of SR and MP that yielded ratios ranging from 189 to 1 (SR : MP)

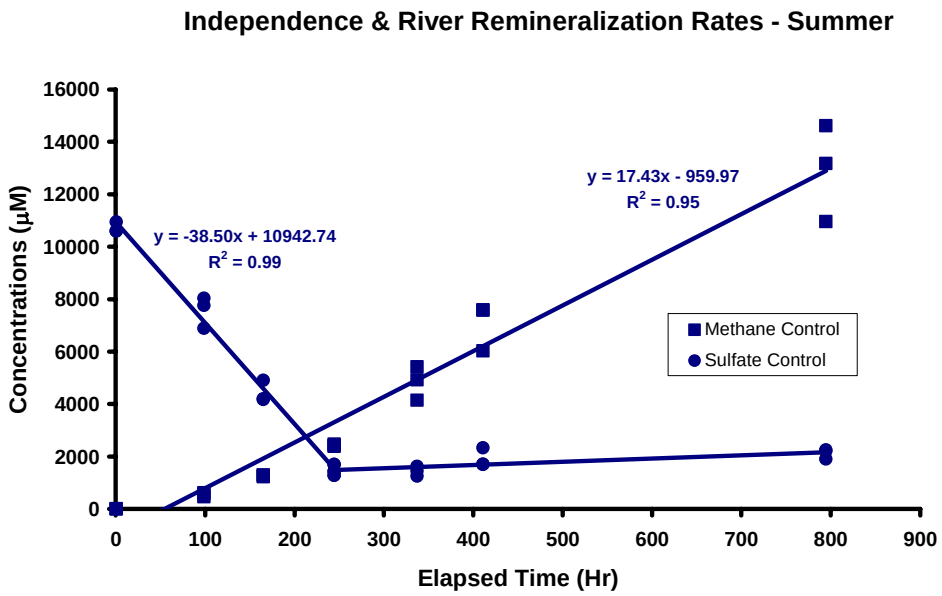


Figure 18. Coexistence of Independence and River remineralization rates during summer. Control MP and SR rates are indicated by the slopes of the lines. R-squared values are given to show the strength of correlation for both rates. Significance was also determined. Both rates are significant ($P \leq 0.05$).

to 0.13 to 1 depending on season for a peatland in Britain. Coexisting rates of comparable magnitudes have not been observed before possibly due to the limited number of studies conducted in estuarine sediments. Figure 19 shows literature MP and SR coexistence ratios compared to the current study.

Percent Organic Matter Content of Sediments and Above-Ground Biomass

In five sites, the percent organic matter content of the dry weight sediments varied seasonally. Percent OM content of the sediments in three sites was lower in winter likely because the sediments had just experienced the warmer temperatures in summer, and thus elevated remineralization rates. Bulk AGB also varied seasonally in all sites containing AGB. There was not much measurable AGB remaining at most sites in the winter because most plants had already died back. In sites where a large amount of AGB was still present, it was generally in the form of dead standing biomass. There was no difference in dry weight OM content of the AGB in half of the sites from summer to winter (Figures 6b and 8), mainly because the same plants were still present.

Below-Ground Primary Production and Carbon Flux from the Sediments

^{137}Cesium data was available for EI, DL, and BR (Renfro 2004) and allowed for a measurement of sediment accumulation at each site. Sediment accumulation rates were determined from gamma spectrometric measurements of ^{137}Cs, which is a thermonuclear bomb fallout isotope (Krishnaswamy et al. 1971, DeLaune et al. 1978, Alongi et al. 2004, Renfro 2004). There was no trend observed for accretion rates (Renfro 2004) in CFMP's sites (Hackney et al. 2005) along a generalized salinity gradient (Figure 20).

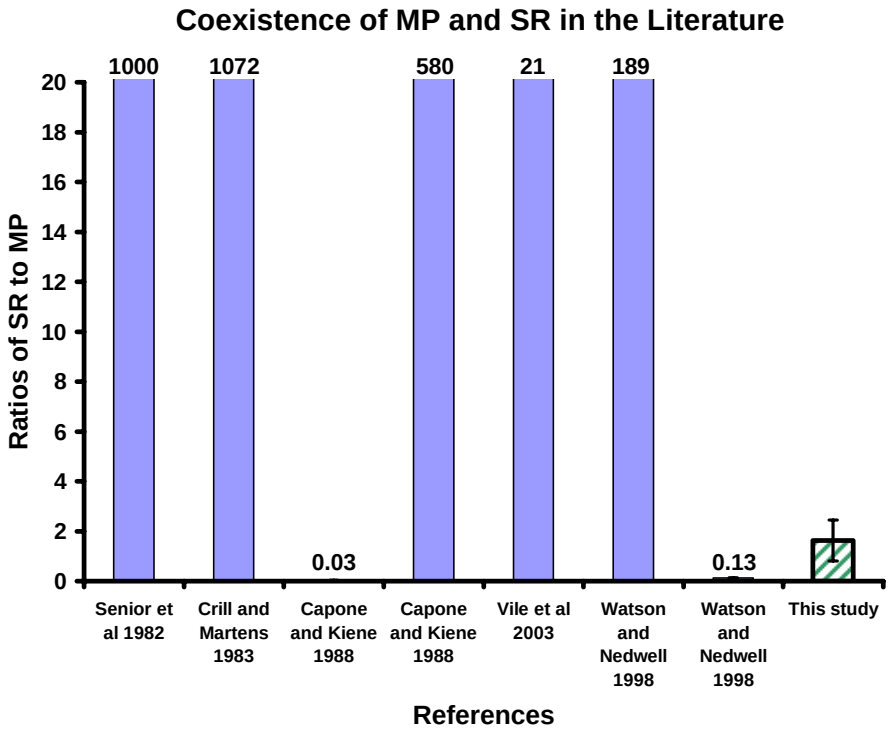


Figure 19. Coexistence of MP and SR in the literature. Ratios of sulfate reduction rates to methane production rates are shown. Ratio values are located over columns of data from the literature that were too large to fit on the graph or too small to be seen on the graph. Literature columns are solid-colored. The SR to MP ratios from the current study are indicated by the striped column. The error bar represents the range of ratios obtained from the current study.

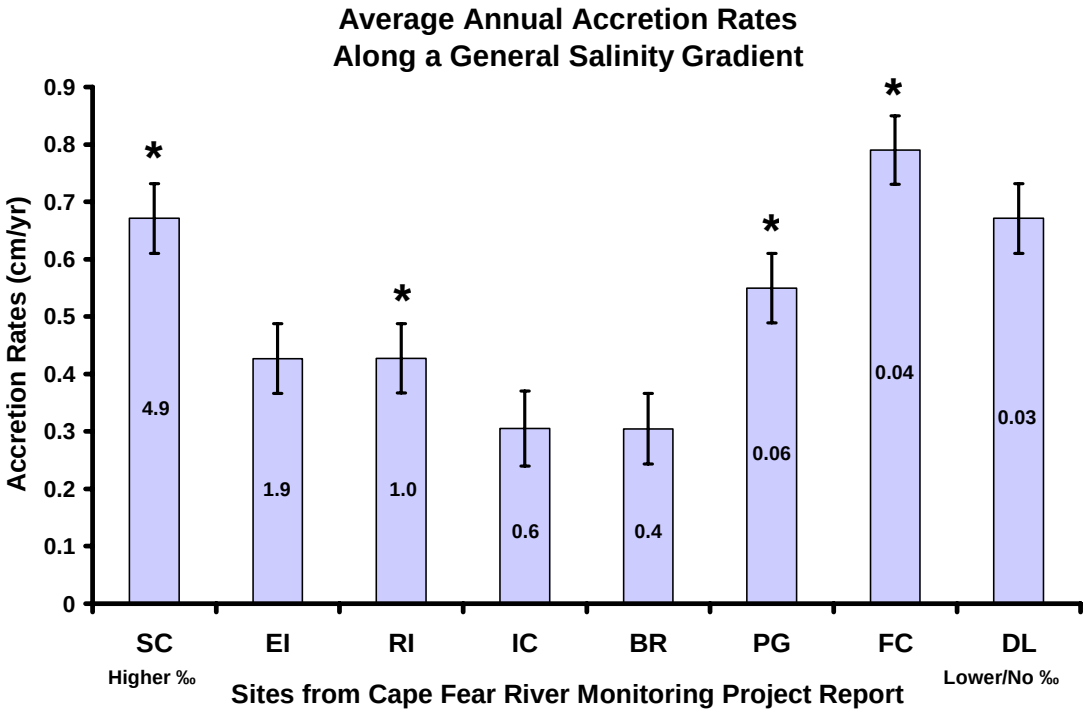


Figure 20. Average annual accretion rates along a general salinity gradient. Error bars represent the range of accretion rates for each site. Higher salinity sites are located on the left side of the graph and decrease in salinity from left (Higher ‰) to right (Lower/No ‰). The general salinity gradient was determined from the salinities measured for the Cape Fear River Monitoring Project and are reported on the columns for each site. These reported salinities are the greatest amount of salinity that each site experienced from summer 2003 and winter 2004 at its respective monitoring station located closest to the Cape Fear River. An asterisk (*) present over a column indicates a site that is located on the Northeast Cape Fear River. If no asterisk is present over a column, that site is located on the mainstem Cape Fear River. Data and site information were obtained from Renfro (2004) and the Cape Fear River Monitoring Project Report (Hackney et al. 2005).

BGPP values using measured ^{137}Cs accumulation rate minimums and maximums and remineralization rates from the current project were calculated for EI, DL, and BR assuming a nine-month growing season. The amounts of carbon flux were also calculated (see Methods). Table 3 shows calculated values of BGPP and carbon flux from the sediments for EI, DL, and BR. BGPP was considered to be new organic carbon (respired plus accumulated carbon) incorporated into the system on an annual basis. Carbon flux from the sediments was AGB produced within and removed from the system on an annual basis.

BGPP and carbon flux values for EI, DL, and BR varied considerably. BGPP values in Table 3 ranged from 970-1293 grams of carbon per meter squared per year (g $C/m^2/yr$) in EI to 890-1068 g $C/m^2/yr$ in DL to 567-855 g $C/m^2/yr$ in BR. These BGPP values were converted to the units grams of biomass/m^2/yr in order to compare them to BGPP literature values in Table 4. In general, BGPP values from this study were less than or similar to values from the literature for saline and estuarine marshes, and greater than or similar to literature values for freshwater wetlands. Therefore, it appears that saline sites in general have the greatest BGPP values, followed by estuarine sites with intermediate BGPP values (e.g. those in this study); and freshwater sites have the lowest BGPP values.

Carbon flux values were highest in EI, intermediate in DL, and lowest in BR. Higher carbon flux from EI sediments was due to this site having the largest amount of AGB available for flux during the growing season. The AGB at DL and BR also fluxed from the system, but there was not as much present at those sites at the beginning. Although total amounts of available AGB were most likely the reason for the highest flux

Table 3. Calculations of below-ground primary production and carbon flux from the sediments. BGPP was considered to be new organic carbon (respired plus accumulated carbon) incorporated into the sediments on an annual basis. Carbon flux from the sediments was AGB produced within and removed from the system on an annual basis. BGPP values were calculated for a nine-month growing season. BGPP values are presented as ranges since minimum and maximum accumulation rates were utilized in the calculations. Units are grams of carbon per meter squared per year.

Sites	**Below-Ground Primary Production (g C/m^2/yr)**	**Carbon Flux (g C/m^2/yr)**
Eagle Island	970-1293	19.1
Dollisons Landing	890-1068	4.5
Black River	567-855	2.4

Table 4. Below-ground primary production values for different types of wetlands. Values were measured and calculated by various techniques including using the Smalley method, littertraps and litterbags, measuring BGB, maximum-minimum estimation procedures, and direct measurements of below-ground respiration, accumulation, and organic matter content. Values are reported in ranges where possible.

Site	Below-Ground Primary Production (g biomass/m^2/yr)	Reference
Marine and Estuarine		
Mississippi *Juncus romerianus* marsh	1360	De la Cruz and Hackney 1977
Massachusetts *Spartina alterniflora* marsh	2500 (short form) 3500 (tall form)	Valiela et al. 1976[a]
North Carolina *Spartina alterniflora* marsh	460 (short form) 503 (tall form)	Stroud 1976[a]
Mississippi *Spartina cynosuroides* marsh	2200	Hackney and De la Cruz 1986
Georgia *Spartina cynosuroides* marsh	3500	Gallagher and Plumley 1979[b]
Georgia *Spartina cynosuroides* marsh	4600	Schubauer and Hopkinson 1984[b]
Cape Fear River, NC - EI	2706-3608[c]	**This study**
Cape Fear River, NC - DL	2483-2980[c]	**This study**
Cape Fear River, NC - BR	1582-2385[c]	**This study**
Freshwater		
Northern bog wetlands	211-513	Reader 1978[d]
Prairie glacial marshes	253-640	van der Valk and Davis 1978[d]
Sedge meadows	134-208	Bernard and Gorham 1978[d]
Tidal marshes	160-223	Whigham et al. 1978[d]
Riverine marshes	296-710	Klopatek and Stearns 1977[d]
Typha spp. marshes	1300 371-954	Keefe 1972[d]

[a] Reference from De la Cruz and Hackney 1977
[b] Reference from Hackney and De la Cruz 1986
[c] BGPP values were converted from g C/m^2/yr in Table 2 to g biomass/m^2/yr for comparison: [BGPP value (g biomass/m^2/yr) = BGPP value (g C/m^2/yr) * 3550 / 12 / 106].
[d] Reference from De la Cruz 1988

at EI, it is also possible that the type of vegetation present could have contributed to the elevated fluxes. It is likely that certain species of AGB are more likely to be removed from a system during the non-growing season than others. It is important to consider the wetland classification of a site and all of the vegetation types present during the growing and non-growing seasons to more accurately interpret carbon budgets in different types of systems.

Pictures of the three sites illustrate the differences in the amounts of AGB at each site during summer and winter (Figure 21). All three sites had more AGB in the summer than in winter. It is most important to note that even though EI still contained the most AGB in the winter, it actually had the largest amount of carbon flux. It should also be noted that woody biomass was not taken into account during the carbon flux calculation, which could have increased flux values if the biomass was exported.

Since much more AGB was present and lost at EI, carbon flux values were very different from those of DL and BR. A large fraction of the bulk AGB (74% for EI, 82% for DL, and 50% for BR) was lost from summer to winter (Figure 8), as well as a large fraction of the dry weight organic matter from the AGB (69% for EI, 80% for DL, and 58% for BR) (Figure 10); indicating a definite loss of organic matter. In EI, where the largest amount of AGB potentially available for flux was located, the carbon flux value was 19.1 g $C/m^2/yr$. DL and BR carbon flux values were 4.5 and 2.4 g $C/m^2/yr$, respectively.

The carbon flux results from EI, DL, and BR were similar in magnitude to many of the literature values (Table 5). In this study, bulk AGB measurements from summer and winter, excluding woody biomass, were utilized to determine carbon flux values. In

a.

b.

c.

d.

e.

f.

Figure 21. Pictures of Eagle Island, Dollisons Landing, and Black River in summer and winter. Woody biomass was not taken into account at these sites. a.,b.) Eagle Island shown in summer and winter. There was a great amount of living AGB in summer and dead standing AGB in winter. c.,d.) Dollisons Landing shown in summer and winter. There was AGB in summer, but not much was found in winter. e.,f.) Black River shown in summer and winter. There was the least amount of AGB found here in the summer compared to the other two sites, and not much in winter.

Table 5. Carbon flux values from different types of wetlands. Values were measured and calculated by various techniques including using nets, littertraps, and litterbags; measuring AGB in different seasons, measuring total organic carbon, total suspended solids, and particulate organic matter in the water column; calculating flux from the literature; and estimating respiration rates. Values are reported in ranges where possible. An asterisk (*) in front of a number indicates a carbon flux value into the system.

Site	Organic Carbon Flux Values (g C/m^2/yr)	Reference
Marine and Estuarine		
Estuary	*125-325	Sutula et al. 2003 and references therein
GA saltmarsh and Duplin River	260-1090	Wang and Cai 2004 and references therein
Saltmarshes	100-200	Nixon 1980 and references therein
North Inlet marsh-estuary, SC	160	Dame and Stilwell 1984
San Francisco Bay tidal marshes	150[a]	Jassby et al. 1993 and references therein
Everglades and Florida Bay	7.1	Sutula et al. 2003
Saltmarsh in France	0.28	Bouchard and Lefeuvre 2000
Cape Fear River Estuary, NC – EI	19.1	**This study**
Cape Fear River Estuary, NC – DL	4.5	**This study**
Cape Fear River Estuary, NC – BR	2.4	**This study**
Freshwater		
British Peatland	34-47	Worrall et al. 2003

[a] Jassby et al. combined tabulations and studies from other authors and took the overall median flux as a representative value for San Francisco Bay tidal marshes. They estimated the flux from tidal marsh habitats adjacent to open water habitats.

other studies, the authors used various techniques to measure carbon flux such as using nets, littertraps, and litterbags; measuring AGB, measuring total organic carbon, total suspended solids, and particulate organic matter in the water column; calculating flux from the literature; and estimating respiration rates.

Literature carbon flux values vary significantly for different types of systems. These variations likely result from differences in tidal influence, elevation, sediment type, salinity, and vegetation composition. They may also vary because of the method by which they were analyzed. For example, the current study's carbon flux values were greater than Bouchard and Lefeuvre's (2000) because western European marshes are generally located at higher elevations (Bouchard and Lefeuvre 2000), and the mineralization of halophyte-derived organic matter almost completely takes place within the marsh system itself (Hemminga et al. 1996) leaving little for flux. EI's carbon flux numbers were an order of magnitude greater than DL's and BR's in this study possibly due to the fact that EI was a marsh, and DL and BR were swamp forests. These results demonstrate that estuarine sediments have a wide range of values. In general, the marine and estuarine systems had larger carbon flux values than freshwater swamps.

Other studies have examined dissolved organic carbon (DOC) flux, however DOC was not considered to be a large percentage of total carbon flux in this study. Published DOC flux values were small compared to total carbon flux. Yelverton and Hackney (1986) estimated net dissolved organic carbon flux at 1.52 g $C/m^2/yr$ via porewater flux in North Carolina saltmarshes located near those in this study.

The current study presents a more comprehensive carbon-budget approach to estimating below-ground primary production within an estuarine wetland and carbon flux

from an estuarine wetland. Many different parameters were directly measured and taken into account at the same time such as below-ground respiration rates, accumulation rates, organic matter contents of the sediments, and seasonal AGB measurements.

Spatial Variation in Biogeochemical Parameters Along a Salinity Gradient

One of the main goals of this project was to determine if any spatial variations existed along a salinity gradient for the following biogeochemical parameters: total carbon remineralized, percent organic matter, and accretion rates. Trends were not observed for sites along a generalized salinity gradient during the different seasons for TCR, sediment dry weight OM content, or accretion rates. Since TCR controls OM content, it follows that no pattern would be observed for OM either. Accretion rates depend on the amount of OM contained in the sediments, and because no pattern was found in OM content, no pattern was found in accretion rates. These results show that there are no clear-cut patterns for biogeochemical parameters along a salinity gradient due to variability in both primary productivity and remineralization rates.

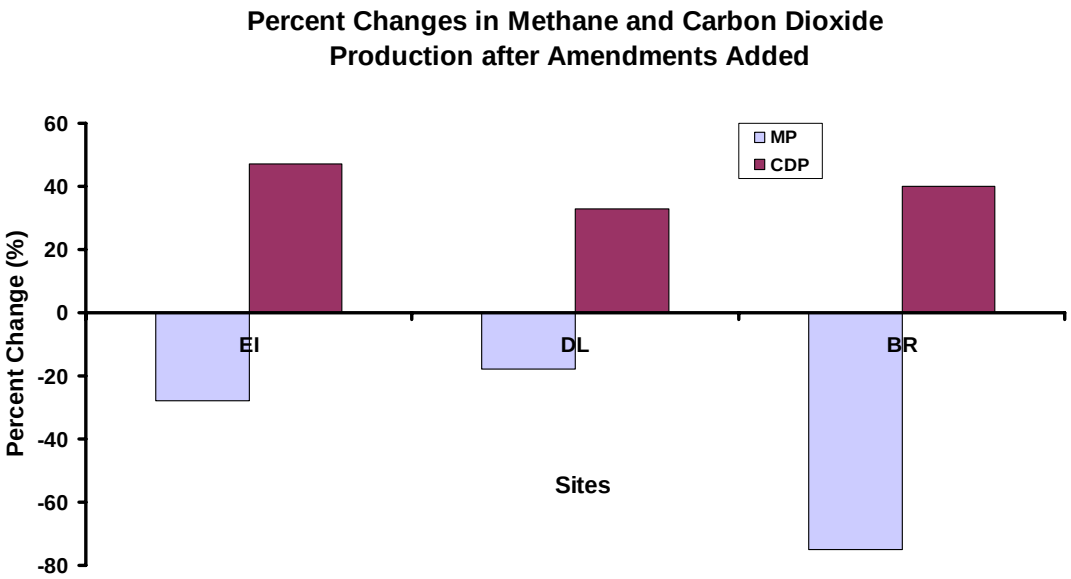
Accretion rates probably do not follow a simple pattern along a salinity gradient because both primary production (PP) and remineralization rates affect the growth of the sediment surface. This study and previous studies have shown that PP increases with salinity, which would tend to increase sediment accretion. However, in general, salinity increases remineralization rates, essentially negating the effect of increased PP. As this study has shown, BR (one of the fresh sites) had the least amounts of PP and remineralization when compared to DL and EI (Table 4 and Figure 12). Also, EI (a more saline site) had the most PP and highest remineralization rates. Therefore, no trends in

accretion rates versus salinity would be expected to be observed between BR and EI (Figure 20).

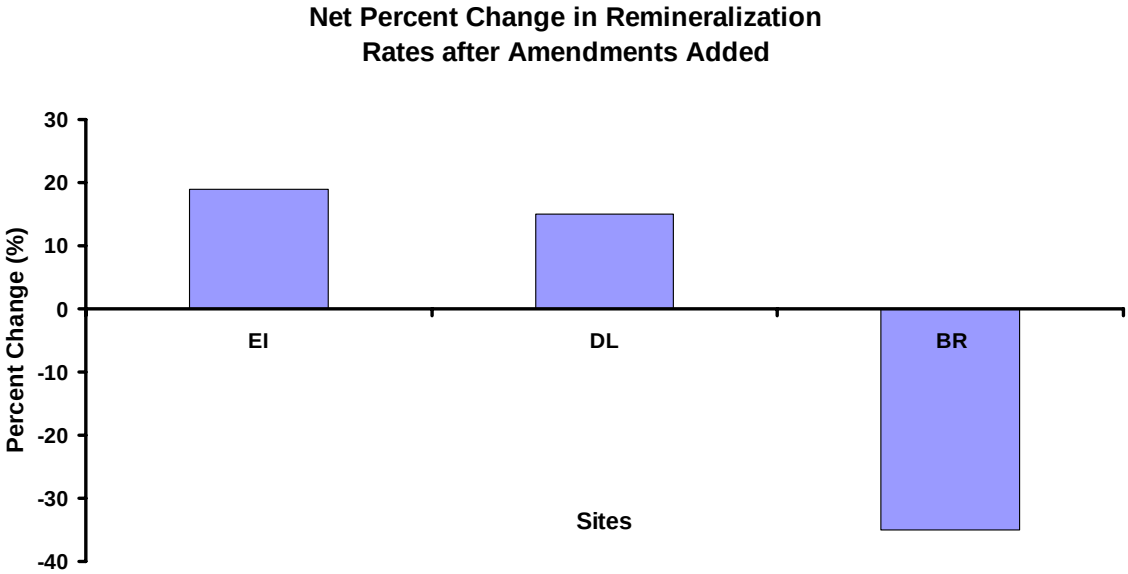
Calculation of Accumulation Decreases with Increased Sulfate Concentrations

Increased remineralization was generally observed when sulfate amendments were added to samples. Based on the percentage by which the remineralization rates increased, a calculation was formulated to determine by what percentage accumulation at these sites would decrease if faced with similar sulfate increases *in situ*. First, MP and CDP rates were compared between control and amended samples for summer (peak growing season) to determine by what percentage rates increased or decreased from control to amended samples when sulfate was added (Figure 22a). Next, the net percentage change of remineralization rates was determined for EI, DL, and BR in summer by adding together the MP and CDP percent changes (Figure 22b). Then, the net percent change was multiplied by the original remineralization rates for each site to determine the amount that their rates increased or decreased after sulfate was added. These numbers were added to their respective original respiration rates. Finally, the new remineralization rates were placed in the remineralization rate portion [RR * SAR * 1.05E-4 * (bulk density of 1.5 cm^3/g)] of the BGPP calculation (see Methods), using minimum and maximum accumulation rates, to calculate a new accumulation rate for each site in mm/yr.

From the calculation, it was determined that EI's accumulation rates decreased (respiration rates increased) by 0.10-0.12 mm/yr, DL's accumulation decreased by 0.08-0.10 mm/yr, and BR's accumulation decreased by 0.01 mm/yr when sulfate



a.



b.

Figure 22. Percent changes in remineralization rates after amendments added. Positive bars represent increases in percent changes, and negative bars represent decreases. a.) MP and CDP rates were compared between control and amended samples for summer (peak growing season) to determine by what percentage rates increased or decreased from control to amended samples when sulfate was added. b.) The net percentage change of remineralization rates was determined for EI, DL, and BR in summer by adding together the MP and CDP percent changes. Note: Scales are different on graphs.

concentrations increased. These numbers can be compared to EI's base ^{137}Cs accumulation rate of 4.2 mm/yr, DL's rate of 6.7 mm/yr, and BR's rate of 3.0 mm/yr (Renfro 2004). Throughout the years, salinities have been gradually increasing in the Cape Fear River estuary. Over 200 years ago, regions of the lower Cape Fear River were generally fresh. Through dredging, snag removal, and the opening of the inlet Snows Cut over the past 100 years, a larger volume of more saline water has been able to be carried further upstream to inundate previously fresh areas (Hackney and Yelverton 1990). Therefore, anthropogenic activities have altered the tidal and salinity regime for the area.

Wetlands are accustomed to the salinities to which they are normally exposed, and when salinities rapidly increase, on a short-term scale they can have an impact on the ecosystem. Not only will they alter the sediments, but they will alter the flora and fauna that reside in the wetlands. Halophobic plants may not be able to withstand increased salinities and may begin to die. This die-back will cause an increase in erosion and decrease in accumulation since no living roots will be present to trap and anchor sediments and to add below-ground volume. Finally, the marsh may begin to subside and become open water, unless other wetland plants begin to colonize it. If the sea level continues to rise rapidly during the wetland transition, this scenario will only be exacerbated.

Currently, however, marshes in the study area appear to be keeping up with sea level rise. The accumulation rate decrease calculation shows that even if sites in the current study are exposed to sulfate concentrations *in situ* on a regular basis, such as those in this study, for the time being their accumulation rates may still out-compete a sea level rise of 2.12 mm/yr that has been reported for these areas (Zervas 2004). However,

the rate of sea level rise is similar to the growth rates of the marshes. Therefore, sea level rise could potentially catch up with and surpass the growth rates of these systems leading to subsidence.

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